ORIGINAL ARTICLE

Detection of Pathologic Prion Protein in the Olfactory Epithelium in Sporadic Creutzfeldt–Jakob Disease

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ABSTRACT

BACKGROUND

Olfactory cortexes and the olfactory tracts are involved in sporadic Creutzfeldt–Jakob disease. We examined peripheral regions of the olfactory sensory pathway, including the olfactory mucosa, to assess whether pathologic infectious prion protein (PrP^{Sc}) is deposited in the epithelium lining the nasal cavity.

METHODS

We studied nine patients with neuropathologically confirmed sporadic Creutzfeldt–Jakob disease. We obtained the brain, the cribriform plate with the attached olfactory mucosa, and the surrounding respiratory epithelium at autopsy. Control samples of nasal mucosa were obtained post mortem or at biopsy from age-matched control subjects and from control patients with other neurodegenerative diseases. The olfactory and respiratory mucosa and the intracranial olfactory system were analyzed by light microscopy, immunohistochemistry, and Western blotting for pathological changes and for deposition of PrP^{Sc}.

RESULTS

In all nine patients with sporadic Creutzfeldt–Jakob disease, PrP^{Sc} was found in the olfactory cilia and central olfactory pathway but not in the respiratory mucosa. No PrP^{Sc} was detected in any of the tissue samples from the 11 controls.

CONCLUSIONS

Our pathological and biochemical studies show that PrP^{Sc} is deposited in the neuroepithelium of the olfactory mucosa in patients with sporadic Creutzfeldt–Jakob disease, indicating that olfactory biopsy may provide diagnostic information in living patients. The olfactory pathway may represent a route of infection and a means of spreading prions.

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Human transmissible spongiform encephalopathies, or prion diseases, include sporadic, inherited, and iatrogenic neurodegenerative disorders[1] characterized by a conformational modification of the host cellular prion protein (PrP^C) into an insoluble and protease-resistant isoform, termed PrP^{Sc}.[2] Prion diseases are neuropathologically characterized by neuronal loss, spongiform degeneration, gliosis, and the deposition of abnormal PrP^{Sc} in central nerve-cell processes and synaptic regions.[3] Sporadic Creutzfeldt–Jakob disease accounts for about 85 percent of all human transmissible spongiform encephalopathies.[4] The disease occurs worldwide and is characterized by the absence of pathogenic mutations in the prion protein (PrP) gene (*PRNP*) and a history of iatrogenic or dietary exposure to infectious sources.[5] Clinical and pathological features of sporadic Creutzfeldt–Jakob disease vary depending on the interaction between the *PRNP* polymorphic codon 129 and distinct conformers of PrP^{Sc}.[6-8] The most common phenotype is characterized by rapidly evolving dementia, myoclonus, complexes of periodic sharp and slow waves on the electroencephalogram, and the presence of 14-3-3 protein (a brain-derived species, which reflects neuronal damage) in the cerebrospinal fluid. Other phenotypes may present with cerebellar or extrapyramidal signs.[9]

Currently, there are no peripheral markers of sporadic Creutzfeldt–Jakob disease for use in the diagnosis of the disease in living patients. Definitive diagnosis requires pathological examination of the brain and the detection of the abnormal PrP isoform by immunohistochemical analysis or immunoblotting.[10] Attention has recently been devoted to the biochemical analysis of components of the eye and optic nerve in sporadic Creutzfeldt–Jakob disease,[11] but little attention has been paid to the olfactory sensory pathway.

We have evidence that the olfactory tracts and cortexes are frequently involved in sporadic Creutzfeldt–Jakob disease, regardless of the genotype at polymorphic codon 129 of *PRNP* and the physicochemical properties of PrP^{Sc}. We sought to determine whether patients with sporadic disease had detectable levels of PrP^{Sc} or deposits of abnormal PrP in the neuroepithelium of the nasal cavity.

METHODS

PATIENTS

We studied nine patients who had been given a diagnosis of definite sporadic Creutzfeldt–Jakob disease on the basis of neuropathological findings and the detection of PrP^{Sc} in the brain on immunohistochemical analysis and Western blotting.[10,12,13] We obtained postmortem specimens from the patients between February 2000 and March 2002, after being notified by their treating physicians in the Veneto region of Italy. Cerebrospinal fluid samples were obtained from all patients, and each sample was tested for 14-3-3 protein.[14] All electroencephalographic tracings were evaluated with the use of standardized criteria.[15]

At the time of notification and during follow-up, the patients were classified according to established diagnostic criteria.[12,16] Probable sporadic Creutzfeldt–Jakob disease was diagnosed in patients who had progressive dementia of less than two years' duration, complexes of periodic sharp and slow waves on the electroencephalogram, and at least two of the following clinical features: myoclonus, visual or cerebellar symptoms (or both), pyramidal or extrapyramidal signs (or both), and akinetic mutism. Patients who fulfilled the clinical criteria but who did not have complexes of periodic sharp and slow waves were classified as having possible sporadic Creutzfeldt–Jakob disease. The duration of the disease was calculated from the onset of neurologic symptoms or signs to death.

DETERMINATION OF THE *PRNP* GENOTYPE

After obtaining written informed consent from the patients' next of kin, we extracted genomic DNA from frozen brain tissues and sequenced the entire coding region of *PRNP*, as described elsewhere.[17]

TISSUE COLLECTION AND PROCESSING

The cribriform plate and the upper portion of the nasal septum, with the attached olfactory mucosa (Fig. 1), were obtained at autopsy from the nine patients with sporadic Creutzfeldt–Jakob disease, five age-matched control subjects without a neurologic disorder, three patients with Alzheimer's disease, two patients with vascular dementia, and one patient with corticobasal degeneration. In all subjects, the interval between death and autopsy ranged from 12 to 32 hours. In each subject, the olfactory mucosa on each side of the nose, easily recognizable because of its yellowish color, was divided in two. One piece was fixed in 4 percent buffered formaldehyde solution and treated for 20 minutes with formic acid — a process that denatures amyloid-protein aggregates and enhances their antigenicity[18] — before being embedded in paraffin for neuropathological examination and immunohistochemical analysis.

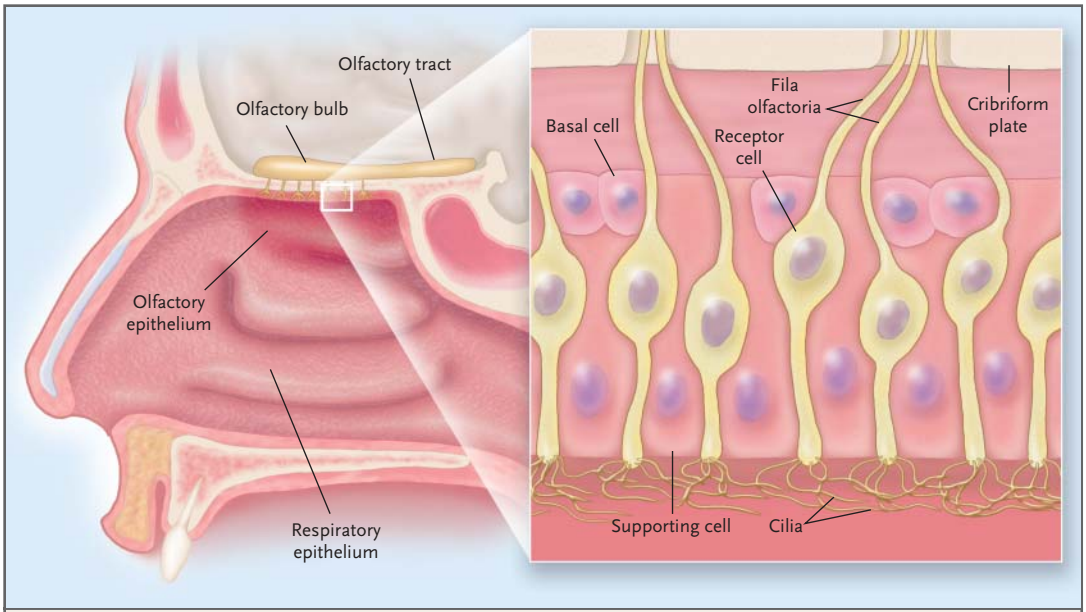

Figure 1. The Peripheral Olfactory System.

Axons from olfactory receptor neurons compose the fila olfactoria, which pass through the cribriform plate of the ethmoid bone to reach the olfactory bulb. In the olfactory mucosa, the dendrites of olfactory receptor neurons extend toward the surface of the epithelium and give rise to olfactory cilia.

The other piece was frozen and stored at −80°C for up to one month until biochemical analyses could be performed. The respiratory mucosa, obtained from contiguous regions of the turbinates, was processed in the same manner. The left olfactory bulb and tract, together with the ipsilateral prepiriform cortex (including the lateral olfactory gyrus and the olfactory uncal region) and the periamygdaloid and entorhinal cortexes, were fixed for neuropathological examination. Contralateral areas were frozen and stored at −80°C until biochemical analyses could be performed. In addition, the optic nerve, frontal, temporal, parietal, and occipital cortexes, hippocampus, thalamus, striatum, and cerebellum were obtained for both pathological and biochemical studies. Specimens obtained for pathological examination were pretreated with formic acid for one hour before being embedded in paraffin.

Paraffin-embedded biopsy specimens of the olfactory mucosa from eight patients with probable Alzheimer's disease,[19] three patients with probable Parkinson's disease (diagnosed according to established criteria),[20] one patient with progressive supranuclear palsy, and one patient with neuropathologically confirmed Lewy-body disease also underwent immunohistochemical analysis for PrP. These specimens were obtained from 1990 to 1992.

IMMUNOCYTOCHEMICAL ANALYSIS

Ten paraffin sections that were 8 μm thick were obtained from each sample of olfactory mucosa and other tissues. Sections were deparaffinized, rehydrated, treated with 98 percent formic acid for 20 minutes at room temperature, and autoclaved at 121°C for 10 minutes in 1.5 mM hydrochloric acid. Sections were rinsed and then incubated overnight at 4°C with monoclonal antibody 3F4 (1:500 dilution), which recognizes nonhuman prion protein and the human prion protein residues 109 to 112.[21] The mouse monoclonal antibody LB509 (1:500 dilution, Zymed Laboratories), which recognizes α-synuclein, expressed by olfactory receptor neurons and basal cells of the olfactory mucosa,[22] was used for the unequivocal identification of these cells when necessary. Subsequent antibody detection involved incubation with a biotinylated goat anti-mouse secondary antibody for one hour (1:500 dilution, Vector Laboratories) at room temperature, followed by incubation with the avidin–biotin–peroxidase complex (Vectastain ABC-Elite kit, Vector Laboratories) according to the manufacturer's instructions. The samples were then stained with 0.06 percent 3,3′-diaminobenzidine as the chromogen and 0.006 percent hydrogen peroxide in 50 mM TRIS buffer, pH 7.6.

IMMUNOBLOT ANALYSIS

From each sample of central nervous system tissue, 100 mg of tissue was homogenized in 0.9 ml of lysis buffer (100 mM sodium chloride, 10 mM EDTA, 0.5 percent nonaethyleneglycol octylphenyl ether, 0.5 percent sodium deoxycholate, and 10 mM TRIS, pH 7.4). For olfactory or respiratory mucosa, each sample of 100 mg of tissue was homogenized in 0.4 ml of lysis buffer. Aliquots were adjusted to a final concentration of 20 or 100 μg of proteinase K (Boehringer Mannheim) per milliliter and incubated at 37°C for 10 to 60 minutes. Samples, equivalent to 300 μg of wet tissue, were resolved on 12 percent gels for sodium dodecyl sulfate–polyacrylamide-gel electrophoresis and then transferred onto polyvinylidene difluoride membrane (Immobilon P, Millipore) for two hours at 60 V. Membranes were blocked with 1 percent nonfat dry milk in 10 mM TRIS, 150 mM sodium chloride, and 0.1 percent Tween-20, pH 7.5, for one hour at 37°C and incubated overnight at 4°C with monoclonal antibody 3F4 (1:10,000 dilution). Blots were developed with an enhanced chemiluminescence system (ECL, Amersham Pharmacia Biotech) and visualized on an autoradiography film (Hyperfilm, Amersham Pharmacia Biotech). Films were scanned with a densitometer (model GS-710, Biorad). The relative amounts of PrP^{C} expressed or PrP^{Sc} distributed were calculated as previously described.[23]

To enhance the detection of PrP^{Sc}, samples of the olfactory and respiratory mucosa were also prepared according to the procedure of Wadsworth et al.[11] Briefly, 100 mg of wet tissue was homogenized in 0.9 ml of 2 percent sarkosyl in phosphate-buffered saline, pH 7.4. Cellular debris was removed by centrifugation at 1000 rpm for 2 minutes, and samples were incubated for 30 minutes at 37°C with constant agitation in phosphate-buffered saline containing 50 U of Benzonase (Benzon nuclease, Merck) per milliliter and 1 mmol of magnesium chloride per liter. Subsequently, samples were adjusted to 0.3 percent sodium phosphotungstic acid (final concentration), incubated at 37°C for 30 minutes, and centrifuged at 14,000 rpm for 30 minutes. The supernatant was saved, and the pellet was dissolved in 20 μl of phosphate-buffered saline, pH 7.4, containing 0.1 percent sarkosyl. The supernatant and the pellet were adjusted to a final concentration of 20 μg of proteinase K per milliliter and incubated at 37°C for 30 minutes.

Table 1. Molecular and Clinical Characteristics of Nine Patients with Sporadic Creutzfeldt–Jakob Disease.*

Patient No.	Age at Onset (yr)/Sex	Codon 129/ PrP^{Sc}†	Signs at Onset	Duration	Clinical Evolution	EEG Findings‡
				mo		
1	72/M	Met/Met 21	Hallucinations	5	Ataxia, myoclonus	PSWs
2	71/F	Met/Met 21	Hallucinations	8	Dementia, ataxia, myoclonus	PSWs
3	73/F	Met/Met 21	Ataxia, dysarthria	16	Dementia	PSWs
4	74/F	Met/Met 21	Dementia	2	Ataxia	PSWs
5	69/F	Met/Met 21	Cortical blindness	5	Dementia, myoclonus	Diffuse slowing
6	59/M	Met/Met 21	Dementia	3	Myoclonus	PSWs
7	55/F	Met/Met 21	Anosmia, hallucinations	5	Dementia	PSWs
8	52/M	Met/Met 21	Dementia	3	Ataxia, myoclonus	PSWs
9	64/F	Val/Val 19	Ataxia	5	Dementia	Diffuse slowing

* All nine patients tested positive for 14-3-3 protein in cerebrospinal fluid.
† Values refer to the molecular mass of the unglycosylated proteinase K–resistant abnormal prion protein (PrP^{Sc}) fragment. Met denotes methionine, and Val valine.
‡ EEG denotes electroencephalogram, and PSWs complexes of periodic sharp and slow waves.

RESULTS

CLINICAL FINDINGS, PRP^{Sc} TYPING, AND CODON 129 GENOTYPING

The age, sex, clinical data, results of electroencephalography and tests for 14-3-3 protein in the cerebrospinal fluid, PrP^{Sc} type, and *PRNP* codon 129 polymorphism of each patient with sporadic Creutzfeldt–Jakob disease are reported in Table 1. In the final stages of the disease, all the patients had dementia with at least two of the diagnostic criteria for sporadic Creutzfeldt–Jakob disease,[12,16] except Patient 1, who rapidly lapsed into akinetic mutism. Patients with complexes of periodic sharp and slow waves on the electroencephalogram were given a clinical diagnosis of probable sporadic Creutzfeldt–Jakob disease, whereas patients without typical electroencephalographic changes were classified as having possible sporadic Creutzfeldt–Jakob disease. None of the patients had a pathogenic mutation in the coding region of *PRNP*.

NEUROPATHOLOGICAL AND IMMUNOHISTOCHEMICAL FINDINGS

Light microscopy was used to confirm the identity of the olfactory and respiratory mucosa on the basis of histologic criteria.[24] In selected specimens, examined in a blinded fashion, the expression of α-synuclein was used to confirm that the olfactory epithelium and the respiratory epithelium had been properly identified. This protein is abundant in the cytoplasm and dendrites of olfactory receptor neurons and absent in ciliated columnar epithelial cells of the respiratory epithelium. Staining with hematoxylin and eosin of sections of the olfactory mucosa from patients with sporadic Creutzfeldt–Jakob disease did not reveal major pathological changes, as compared with corresponding tissues from controls with and those without other neurodegenerative disorders. Immunostaining for PrP was negative in the olfactory epithelium of all postmortem and biopsy specimens from controls (Fig. 2A). In contrast, immunohistochemical analysis revealed marked deposition of PrP in the cilia of olfactory receptor neurons and a faint PrP immunoreactivity in basal cells of the olfactory epithelium from all patients with sporadic Creutzfeldt–Jakob disease (Fig. 2B and 2C). Conversely, the respiratory epithelium did not stain for PrP (data not shown). In the brains of patients with sporadic Creutzfeldt–Jakob disease, there was selective deposition of PrP in olfactory-bulb glomeruli (Fig. 2D), olfactory tracts (Fig. 2E), and primary olfactory cortexes (Fig. 2F).

EXPRESSION OF PRP^C IN THE OLFACTORY AND RESPIRATORY MUCOSA

Western blot analysis of samples of olfactory and respiratory mucosa from controls showed that both

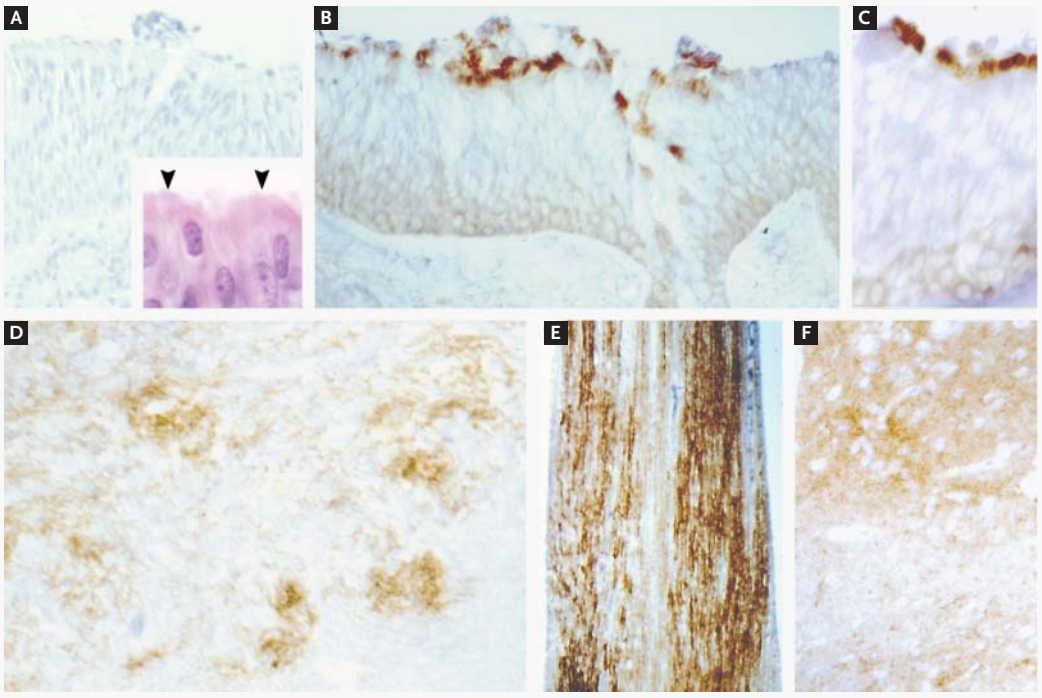

Figure 2. Immunostaining for Prion Protein in Tissues from Controls and from Patients with Sporadic Creutzfeldt–Jakob Disease.

There is no prion protein immunoreactivity in the olfactory mucosa from a 68-year-old control subject (Panel A, ×240); the inset (hematoxylin and eosin, ×536) shows an adjacent section, in which the arrowheads point to the olfactory cilia. PrP deposition is observed in the olfactory cilia and basal cells of a specimen of olfactory mucosa from Patient 2 with sporadic Creutzfeldt–Jakob disease (Panel B, ×240); the pattern of PrP immunostaining is discontinuous, probably because of the nonuniform presence of olfactory cilia. In tissue specimens from Patient 4, PrP is present in the olfactory epithelium (Panel C, ×412), olfactory-bulb glomeruli (Panel D, ×262), olfactory tract (Panel E, ×120), and olfactory uncal region (Panel F, ×100).

tissues constitutively expressed PrP^C. PrP^C was detected mainly in a highly glycosylated form and had a slightly faster rate of migration than that derived from the brain (Fig. 3). The levels of PrP^C expression in the olfactory and respiratory mucosa were approximately 20 percent and 13 percent, respectively, of that in brain.

DETECTION OF PRP^{Sc} IN OLFACTORY MUCOSA

After treatment with proteinase K at a concentration of 20 μg per milliliter for 20 or 10 minutes, PrP^{Sc} was undetectable on immunoblot analysis of homogenates of olfactory and respiratory mucosa from patients with sporadic Creutzfeldt–Jakob disease and from controls (Fig. 4A). To increase the sensitivity of the method of PrP^{Sc} detection, we tested the ability of sodium phosphotungstic acid, which binds efficiently to hamster and human PrP^{Sc},[11,25] to precipitate PrP^{Sc} from the nasal mucosa of patients with sporadic Creutzfeldt–Jakob disease. Western blot examination of the supernatant from homogenates of olfactory mucosa showed a major PrP band migrating at approximately 33 kD, which was digested by proteinase K (Fig. 4B, lanes 1 and 2). By contrast, Western blot examination of the insoluble fraction, recovered in the pellet, showed four main PrP-reactive bands in the range of approximately 23 to 35 kD. Treatment of the insoluble fraction with proteinase K generated three major proteinase K–resistant PrP^{Sc} bands (Fig. 4B, lanes 3 and 4), with an unglycosylated fragment of approximately 22 kD in all patients who were homozygous for methionine and of approximately 21 kD in the patient who was homozygous for valine at codon 129. In four patients, the olfactory mucosa contained levels of PrP^{Sc} equivalent to approximately 3 percent of those found in the olfactory-bulb homogenates. PrP^{Sc} immunoreactivity was not detected either in the respiratory mucosa from patients with sporadic Creutzfeldt–Jakob disease or in the olfactory and respiratory mucosa from control subjects.

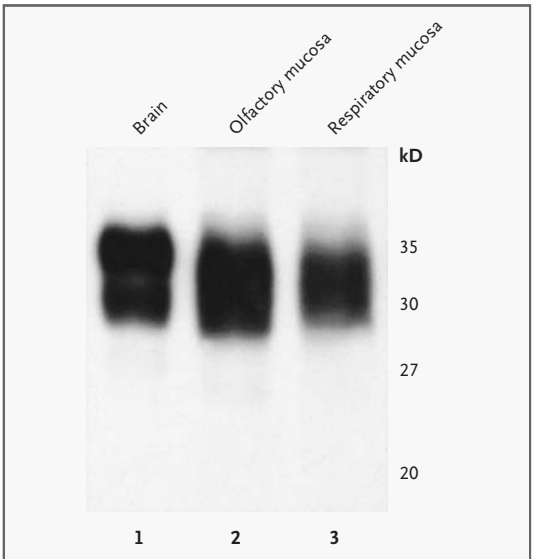

Figure 3. Expression of Cellular Prion Protein in Olfactory and Respiratory Mucosa from Control Subjects.

Western blotting with the 3F4 monoclonal antibody shows the level of expression of cellular prion protein (PrP^C) in 5.3 μg of brain homogenate (lane 1), 21 μg of olfactory mucosa (lane 2), and 16 μg of respiratory mucosa (lane 3). Brain PrP^C is detected as two major bands corresponding to diglycosylated and monoglycosylated isoforms; olfactory PrP^C and respiratory PrP^C are detected as highly glycosylated forms, which migrate slightly faster than brain PrP^C.

REGIONAL DISTRIBUTION OF PRP^{Sc} IN OLFACTORY AREAS, NEOCORTEX, SUBCORTICAL NUCLEI, AND CEREBELLUM

After treatment with proteinase K at a concentration of 100 μg per milliliter for one hour, patients who were homozygous for methionine had the largest amounts of PrP^{Sc} in the neocortex, as expected, with smaller amounts in the thalamus, basal ganglia, and cerebellum (Fig. 5A). The amount of PrP^{Sc} was very low in the hippocampus, whereas the entorhinal and olfactory cortexes had relatively large quantities of PrP^{Sc}. In addition, in all nine patients, PrP^{Sc} was detected in the olfactory bulb and tract. The protein was undetectable in samples from the prechiasmatic optic nerve.

Of particular interest was the distribution of PrP^{Sc} in the single patient who was homozygous for valine at codon 129 and had a short duration of disease (Table 1). In this patient, levels of the pathologic protein were undetectable in the occipital cortex, minimal amounts were found in the frontal cortex, and the largest amounts of PrP^{Sc} were recovered in the cerebellum and subcortical nuclei (Fig. 5B). In contrast to the absence of the protein or low levels of the protein in the neocortex, moderate amounts of PrP^{Sc} were found in the olfactory bulb, tract, and cortexes.

DISCUSSION

We found that in patients with sporadic Creutzfeldt–Jakob disease, pathologic prion protein is selectively deposited within the neuroepithelium of

the olfactory mucosa, although cellular prion protein is evenly distributed in both the olfactory and respiratory mucosa. These results indicate that factors other than constitutive expression of PrP^{C} determine whether PrP is deposited at the level of the olfactory cilia but not at the level of the respiratory cilia. Our findings, as well as our own unpublished observations, provide evidence that the olfactory sensory pathway is involved in sporadic Creutzfeldt–Jakob disease.

The detection of PrP^{Sc} on immunocytochemical analysis and Western blotting is important for the diagnosis of prion diseases.[26] PrP^{Sc} is an accurate marker for the infectious agent, and its presence suggests infectivity. The relatively low level of PrP^{Sc} that we detected in the olfactory mucosa of patients with sporadic Creutzfeldt–Jakob disease (about 3 percent of that in the olfactory bulb) may be related to the characteristics of the olfactory epithelium. The main factors that may limit the accumulation of PrP^{Sc} in the olfactory epithelium are the low level of constitutive expression of PrP^{C}, as compared with that in brain tissues, and the short life span (a few weeks) of the olfactory receptor neurons, which are continually expelled in the upper nasal cavity as they are replaced by basal neurons. The highly selective deposition of PrP^{Sc} in olfactory receptor neurons may indicate affinity mechanisms or interactions with one or more of the thousands of receptor proteins expressed in the apical portion of the cilia.[27]

Besides providing evidence of the deposition of PrP^{Sc} in the peripheral tissues of patients with sporadic Creutzfeldt–Jakob disease, our data have important implications regarding both the in vivo diagnosis of the condition and the risk of infection from living patients. Since we examined postmortem samples of olfactory mucosa, we could not pinpoint the stage at which PrP^{Sc} deposition occurs in sporadic Creutzfeldt–Jakob disease. However, the detection of PrP^{Sc} in olfactory tissue in patients with disease of short duration suggests that the PrP^{C} may be converted to PrP^{Sc} at the same time as it is in the central nervous system, where the formation of PrP^{Sc} always precedes the appearance of lesions.[28] In one patient the disease presented with anosmia, and there is a report of variant Creutzfeldt–Jakob disease presenting with loss of taste and smell.[29] In the patient with the ataxic variant of relatively short duration, PrP^{Sc} accumulated in the olfactory paleocortex, despite the absence of accumulation in the hippocampus and neocortex. Given the small number of patients analyzed in this study, our results should be viewed with caution, but taken together they suggest that the olfactory system is involved at an early stage in sporadic Creutzfeldt–Jakob disease. In contrast, the retina and optic nerves do not have

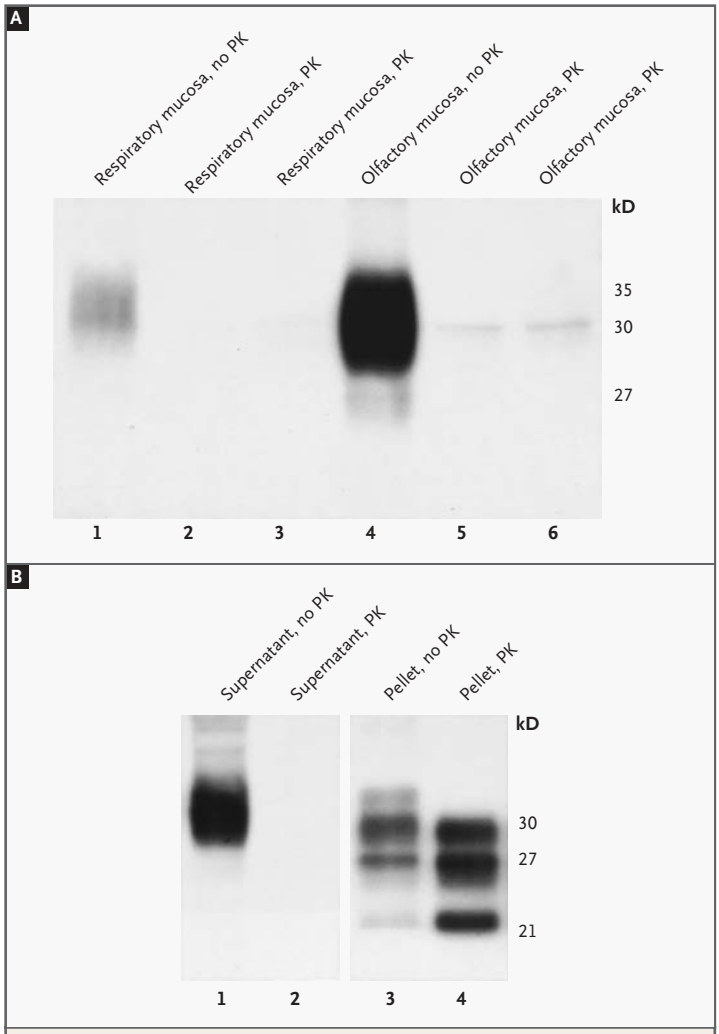

Figure 4. Western Blot Analysis (Panels A and B) of Respiratory and Olfactory Mucosa from Patients with Sporadic Creutzfeldt–Jakob Disease for the Presence of Abnormal Prion Protein (PrP^{Sc}) after Incubation with Proteinase K (PK).

In Panel A, lanes 1 and 4 show untreated homogenates of respiratory mucosa (15 μg) and olfactory mucosa (47 μg), respectively, from Patient 2. Samples of approximately 300 μg of respiratory mucosa (lanes 2 and 3) and olfactory mucosa (lanes 5 and 6) were digested with 20 μg of proteinase K per milliliter, and no protease-resistant PrP^{Sc} was found; a nonspecific band is apparent in lanes 5 and 6. The 3F4 monoclonal antibody was used as a probe. In Panel B, immunoblot analysis of the supernatant (lanes 1 and 2) and of the sodium phosphotungstic acid pellet (lanes 3 and 4), before (lanes 1 and 3) and after (lanes 2 and 4) proteinase K digestion, showed the presence of protease-resistant PrP^{Sc} in olfactory mucosa from Patient 6. The pellet was obtained from 100 mg of tissue (wet weight).

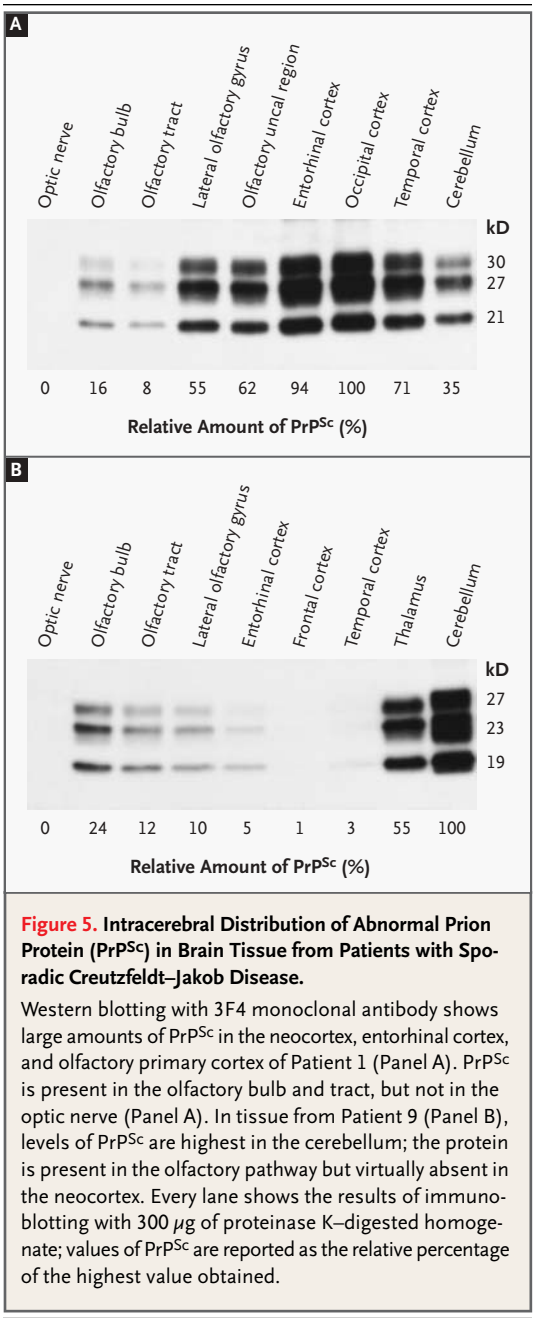

Figure 5. Intracerebral Distribution of Abnormal Prion Protein (PrP^{Sc}) in Brain Tissue from Patients with Sporadic Creutzfeldt–Jakob Disease.

Western blotting with 3F4 monoclonal antibody shows large amounts of PrP^{Sc} in the neocortex, entorhinal cortex, and olfactory primary cortex of Patient 1 (Panel A). PrP^{Sc} is present in the olfactory bulb and tract, but not in the optic nerve (Panel A). In tissue from Patient 9 (Panel B), levels of PrP^{Sc} are highest in the cerebellum; the protein is present in the olfactory pathway but virtually absent in the neocortex. Every lane shows the results of immunoblotting with 300 μg of proteinase K–digested homogenate; values of PrP^{Sc} are reported as the relative percentage of the highest value obtained.

PrP^{Sc} deposition,[11] though the retina may be vulnerable.[30] Our findings suggest that a biopsy of olfactory tissue in patients with suspected sporadic Creutzfeldt–Jakob disease may provide a means of early clinical diagnosis and a way to assess the potential effectiveness of anti-prion compounds when they become available.[31]

The presence of PrP^{Sc} in the nasal cavity of patients with sporadic Creutzfeldt–Jakob disease also raises the question of whether these patients represent a source of infection or have been infected through the olfactory route. Human prion diseases can be transmitted through contaminated electrodes and neurosurgical instruments,[5] and recent work demonstrates that prions are easily and tightly bound by stainless-steel surfaces.[32] Following the appearance of variant Creutzfeldt–Jakob disease, which is caused by the same prion strain as that responsible for cattle bovine spongiform encephalopathy,[33] and the demonstration of infectious prion protein in human tonsillar tissues,[34] precautions to avoid iatrogenic transmission have been undertaken in the United Kingdom. Similarly, the demonstration of a rather high level of infectivity in gingival and dental-pulp tissues from hamsters with experimentally induced scrapie has aroused concern about the risk of transmission in humans through dental work.[35] Our findings also call attention to the possibility that endoscopic and surgical procedures involving the upper vault of the nasal cavity represent a risk factor for prion spreading. However, we know of no cases of disease transmission by this route.

It remains unknown whether the olfactory epithelium represents a gate of entry or only a site of infection during the propagation of prions in neural circuits. In the light of our findings, the hypothesis that sporadic Creutzfeldt–Jakob disease might be initiated by somatic mutations in *PRNP*[36] is intriguing. It should be emphasized that neither the basal epithelium of the olfactory mucosa nor the olfactory bulb is a postmitotic tissue; instead they represent well-established sites of ongoing peripheral and central neurogenesis.[37] Thus, if sporadic Creutzfeldt–Jakob disease is caused by somatic mutations of *PRNP* in the neurons of adults, then the olfactory epithelium and the olfactory bulb are candidate sites for such events.

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