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ORIGINAL ARTICLE

Prion Disease Induces Alzheimer Disease–Like Neuropathologic Changes

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Abstract

We examined the brains of 266 patients with prion disease (PrionD) and found that 46 patients (17%) had Alzheimer disease (AD)–like changes. To explore potential mechanistic links between PrionD and AD, we exposed human brain aggregates (BrnAggs) to a brain homogenate from a patient with sporadic Creutzfeldt-Jakob disease and found that neurons in human BrnAggs produced many β-amyloid (Aβ; Aβ42) inclusions, whereas uninfected control-exposed human BrnAggs did not. Western blot analysis of 20 pooled Creutzfeldt-Jakob disease–infected BrnAggs verified Aβ42 levels higher than those in controls. We next examined the CA1 region of the hippocampus from 14 patients with PrionD and found that 5 patients had low levels of scrapie-associated prion protein (PrP^{Sc}), many Aβ42 intraneuronal inclusions, low apolipoprotein E-4 (APOE-4), and no significant nerve cell loss. Seven patients had high levels of PrP^{Sc}, low Aβ42, high APOE-4, and 40% nerve cell loss, suggesting that APOE-4 and PrP^{Sc} together cause neuron loss in PrionD. There were also increased levels of hyperphosphorylated tau protein (Hτ) and Hτ-positive neuropil threads and neuron bodies in both PrionD and AD groups. The brains of 6 age-matched control patients without dementia did not contain Aβ42 deposits; however, there were rare Hτ-positive threads in 5 controls, and 2 controls had few Hτ-positive nerve cell bodies. We conclude that PrionD may trigger biochemical changes similar to those triggered by AD and suggest that PrionD is a disease involving PrP^{Sc}, Aβ42, APOE-4, and abnormal tau.

Key Words: Aβ42, Alzheimer disease, Codon 129, Creutzfeldt-Jakob disease, Female-to-male ratio, PrP^{Sc}, Tau hyperphosphorylation.

INTRODUCTION

As part of our collaborative studies with Dr. Stanley B. Prusiner on the biology and treatment of prion disease (PrionD) and other neurodegenerative diseases, we have collected and analyzed the brains of 266 patients with PrionD, including 240 patients with sporadic Creutzfeldt-Jakob disease (sCJD), 19 patients with familial Creutzfeldt-Jakob disease (fCJD), and 7 patients with Gerstmann-Sträussler-Scheinker disease (GSS). Prion disease and Alzheimer disease (AD) may share certain pathogenic mechanisms. In 1998, Hainfellner et al (1) reported a 10.9% overlap of AD in 110 cases of Creutzfeldt-Jakob disease (CJD). They concluded that the coexistence of AD-type pathology with CJD most probably represented coincidental events. On the other hand, Lauren et al (2) reported that cellular prion protein (PrP^{C}) is a high-affinity receptor of β-amyloid (Aβ; Aβ42) oligomers in AD and that binding to PrP^{C} mediates the deleterious effects of Aβ42. Parkin et al (3) showed that PrP^{C} inhibits the β-secretase (BACE-1) cleavage of amyloid precursor protein (βAPP), which is the first step in the formation of Aβ40 and Aβ42. Kovacs et al (4) reported that fCJD associated with the E200K *PRNP* mutation forms a complex proteinopathy consisting of scrapie-associated prion protein (PrP^{Sc}), tau, α-synuclein, and deposition of Aβ. They also reported that Aβ deposits may be detected in sCJD.

Here, we investigated how the replacement of PrP^{C} with PrP^{Sc} may promote Aβ42 production and how PrionD might induce AD-like pathologic changes in some patients. First, we determined the number of definite AD or early (incipient) AD cases (defined by the presence of extracellular Aβ42 neuritic plaques) among our 266 cases of PrionD. Next, we wanted to know whether intracellular Aβ42 peptide is formed in neurons and glial cells in the absence of Aβ42 plaque formation in PrionD. Because abnormal hyperphosphorylated tau protein (Hτ) and Aβ42 neuritic plaques define AD, we wanted to know whether Hτ is formed in the brains of patients with PrionD. We report the concomitant occurrence of neuropathologic findings consistent with PrionD and AD, suggesting that Aβ peptides and abnormal tau are induced in PrionD. For controls, we used 6 age-matched patients with no neurologic diseases. Other control data were obtained from the literature and included reports of examinations of the brains of a large number of nondemented patients (5–9).

To test the interaction of PrP^{C}, PrP^{Sc}, Aβ42, and abnormal tau, we used an in vitro cell model of brain aggregates (BrnAggs). After 20 days in culture, these BrnAggs were found to contain mature neurons with dendrites, axons, and synapses, and other cellular CNS elements, including astrocytes, oligodendrocytes, and microglia. In a previous study in

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mice, we showed that the susceptibility of BrnAggs to prions was similar to the susceptibility of BrnAggs to in vivo disease and that BrnAggs provided a clear view of the distribution of PrP^{Sc} in the plasma membrane and subcellular structures (10).

MATERIALS AND METHODS

Patients

We examined 266 consecutive cases of PrionD in the Neuropathology Research Laboratory of the Department of Pathology at University of California San Francisco (UCSF). We also studied 127 AD cases from the files of the Neuropathology Unit. As controls, we examined the brains of 6 age-matched (3 men and 3 women; aged 54–69 years) nondemented individuals.

Estimates of Gross Brain Abnormalities

Brains were characterized by degree of atrophy (i.e., *none*, *mild*, *moderate*, or *severe*) based on brain weight and visual inspection of the cerebral cortex. Atrophy of the hippocampus was scored as positive if the inferior horn was dilated and the size of the hippocampus was smaller than normal on visual inspection. Protrusion of the lateral ventricles (0.2–1.5 cm) into the frontal lobe in a coronal section taken immediately rostral to the temporal poles qualified as mild hydrocephalus; extensive rounding and dilation of the anterior horn, body, and inferior horn of the lateral ventricle and third ventricle were considered as severe hydrocephalus. Depigmentation of the locus coeruleus (LC) and substantia nigra was also evaluated.

Histoblot Technique

Cryostat sections of brain samples were prepared from fresh frozen tissue. Coronal (full and half) sections of fresh frozen human brains were cut (25 μm thick) with a Leica Cryopolycut microtome (International Medical Equipment Inc, San Marcos, CA). Routine frozen sections of the brain (1–2 × 3 inches) were cut (10 μm thick) with a Microm HM505 cryostat (Carl Zeiss, Thornwood, NY) and pressed onto the nitrocellulose membrane. The resulting histoblots were treated with proteinase K to eliminate PrP^{C} and with 3 mol/L of guanidinium to denature PrP^{Sc} for immunohistochemical staining of protease-resistant PrP^{Sc}, which is diagnostic of PrionD (11, 12). The same technique, without the denaturation step, was used to stain for protease-resistant Aβ peptides characteristic of AD (13). The histologic diagnoses of incipient and definite AD were established by semiquantitative estimates of the number of neocortical Aβ42 neuritic plaques using the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) criteria (14).

Neurohistology and Molecular Biology

Brain samples were fixed in 10% buffered formalin and embedded in paraffin using standard procedures. Degree of vacuolation as a percentage of gray matter area, pattern, and laminar distribution of vacuolation (assessed with hematoxylin-and-eosin stain) were estimated. Hematoxylin-and-eosin–stained tissue was also evaluated for the presence of other lesions such as infarcts and Lewy bodies. Fresh frozen and formalin-fixed tissues were sent to the National Prion Disease Surveillance Center for genetic testing of *PRNP* gene mutations and codon 129 polymorphism and for Western blot analysis of PrP^{Sc} phenotypes.

Immunohistochemistry of Human Brains

Brain sections were cut (8 μm thick), deparaffinized, and processed for immunohistochemistry. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol. Nonspecific antibody binding sites were blocked with 10% normal horse serum. For detection of human PrP^{Sc}, we used mouse 3F4 antibody (1:1000) and HuM-P antibody conjugated to horseradish peroxidase (1:10000) (gifts from Dr. Stanley B. Prusiner, UCSF, San Francisco, CA). The intensity of PrP^{Sc} immunostaining was scored semiquantitatively as *mild* (1+), *moderate* (2+), *moderately severe* (3+), or *severe* (4+).

Abnormal Hτ was detected with mouse AT8 antibody (1:250), which is specific for phosphorylated paired helical filamentous tau Ser202/Thr205 (cat no. MN1020B; Life Technologies, Carlsbad, CA). We stained Aβ42 with 4G8 monoclonal antibody (1:500) specific for Aβ42 at epitopes 12 to 24 (cat no. SIG-39220; BioLegend Co [formerly Covance Antibody Products], San Diego, CA). Cortical Lewy bodies were detected with mouse monoclonal α-synuclein antibody (1:250) (LB509, cat no. Ab27766; Abcam, Cambridge, MA). Sections to be stained with 3F4 and AT8 antibodies were subjected to hydrolytic autoclaving for 10 minutes at 121°C in citrate buffer. Sections to be stained with 4G8 monoclonal antibody were immersed in formic acid for 5 minutes. Tissue stained with α-synuclein antibodies was not pretreated. All sections were incubated with primary antibodies overnight at room temperature and with biotinylated horse anti-mouse secondary antibody (1:200) (cat no. BA-2000; Vector Laboratories Inc, Burlingame, CA) for 30 minutes at room temperature. Antibody binding was detected using a Vectastain ABC kit (PK-4000) and visualized with 3,3′-diaminobenzidine (DAB peroxidase substrate kit SK-4100; Vector Laboratories Inc).

Scoring Cortical Hτ

Hyperphosphorylated tau protein immunopositivity in the LC/raphe nuclei (RN) of nerve cell bodies, neuropil threads, and dystrophic neurites was scored (from 0 to 4+) and multiplied by the total area of the LC/RN involved. Total Hτ load in the medial temporal lobe (MTL) was estimated by multiplying Hτ score by the extent of affected layers in the cortex (×1 if Hτ was found in 2–3 layers; ×2 if Hτ was found in all 6 layers). Final scores were multiplied by the number of regions in the MTL in which Hτ was found, which varied from 1 to 6 depending on the number of affected regions (hippocampus, subiculum, presubiculum, entorhinal cortex, transentorhinal cortex, and inferior temporal cortex).

Brain Aggregates

Mouse BrnAggs (Mo BrnAggs) were prepared from E15 day gestation embryos obtained from pregnant transgenic (tau-P301L)4510 mice (gift from Dr. George Carlson, McLaughlin Research Institute, Great Falls, MT). Fetal brains were genotyped by polymerase chain reaction for the presence of tau(P301L), and the calmodulin kinase II promoter system was used to construct transgenic mice (15, 16). Human fetal brain tissues at 15 to 17 weeks of gestation were obtained from Advanced Bioscience

Resources Inc (Alameda, CA) and used to prepare cell cultures of human BrnAggs (Hu BrnAggs). All experiments involving human brain tissue were performed at Biohazard Safety Level 3 in accordance with our Biologic Use Authorization.

Brain aggregates were prepared as previously described (10). Briefly, mouse and human brain cells were dissociated through 2 nylon meshes. After 2 washes with DMEM H21 containing glucose (12 g/L), fungizone (2.5 mg/L), and gentamicin (50 mg/L), dissociated neural cells were resuspended in a modified growth medium DMEM H21 containing glucose (6 g/L), gentamicin (50 mg/L), fungizone (2.5 mg/L), and 10% fetal bovine serum at a density of 1×10^7 cells/mL. Four milliliters of cells was placed in 25-mL Delong flasks and kept at constant rotation (37°C, 10% CO_2). The next day, 1 mL of exchange medium consisting of DMEM H21 supplemented with glucose 6 g/L, gentamicin 50 mg/L, and 15% fetal bovine serum was added to the flask. After 2 to 3 days, rotated BrnAggs were transferred to 50-mL Delong flasks to which 5 mL of exchange medium was added, for a total of 10 mL. The exchange medium was refreshed every 2 to 3 days by removing 5 mL of conditioned medium and replacing it with 5 mL of fresh exchange medium. For Mo BrnAggs, after 6 to 8 days in culture, the growing and fusing BrnAggs were transferred to a 24-well culture plate, which was also maintained in constant rotation. For Hu BrnAggs, this process was performed at approximately 12 to 14 days. The medium was changed every 2 to 3 days by discarding 500 μL of conditioned medium and replacing it with an equal volume of exchange medium.

Exposure of BrnAggs to Prions

After 15 days in culture, Mo BrnAggs were exposed for 10 days to Rocky Mountain Laboratories prions (1:50 dilution) derived from scrapie-infected CD1 mouse brains. After 41 days in culture, Mo BrnAggs were harvested and analyzed by immunohistochemistry. Paraffin-embedded sections were stained for phosphorylated tau with mouse anti-tau13 antibody (1:500, cat no. ab24634; Abcam), mouse anti-tau CP13 antibody (1:250; a gift from Dr. Peter Davies, Albert Einstein College of Medicine of Yeshiva University, New York, NY), or the secondary antibody goat anti-mouse Alexa Fluor 488 (1:200, cat no. A11029; Life Technologies). Three to 4 stacks of approximately 40 (1 μm thick) serial sections were captured by confocal microscopy (LSM 510; Zeiss, Jena, Germany). Whole BrnAggs were examined throughout by a fluorescence microscope with a 40× lens (DM IRB; Leica, Wetzlar, Germany).

For experiments with Hu BrnAggs, the cells were exposed to a human brain homogenate (1:50 dilution) containing either sCJD(VV2) made from the thalamus of sCJD(VV2) cases or normal brain (gift from Dr. William Seeley, UCSF Memory and Aging Center, San Francisco, CA) after 2 days in culture. Treatments were continued for up to 20 days in culture. Human BrnAggs were harvested after 35 days. Twenty BrnAggs were pooled and analyzed for PrP^{Sc} by Western blot analysis using anti–prion protein (PrP) HuM-P horseradish peroxidase (1:10000) antibody, Aβ1–42 (Aβ42)–specific antibody (1:500, SIG-39142; BioLegend Co [formerly Covance Antibody Products]), and anti-tau oligomeric antibody (1:1000, ABN454; Millipore, Temecula, CA) overnight at room temperature.

For detection of Aβ42, 5 Hu BrnAggs exposed to sCJD prions and 2 control BrnAggs not exposed to prions were fixed with 4% paraformaldehyde for 1 hour. The BrnAggs were embedded in paraffin, cut (8 μm), and mounted on glass slides. After deparaffinization, they were submerged in formic acid for 5 minutes for antigen retrieval and incubated with Aβ1–42 (Aβ42)–specific antibody (1:500) overnight at room temperature. The secondary antibody, goat anti-mouse Alexa Fluor 488 (A11029; Life Technologies) conjugated to fluorescein isothiocyanate, was applied for 2 hours. The slides were coverslipped in mounting medium containing DAPI (H-1200; Vector Laboratories Inc), analyzed, and photographed with a Leica DM IRB fluorescence microscope.

Quantification of Western Blot Analysis

Levels of Aβ were quantified with BioQuant Life Science software (Bioquant Image Analysis Corp, Nashville, TN).

Statistical Analysis

Statistical analysis of human brain weights and Mo BrnAggs was performed using Student *t*-test. Chi-squared test was used to compare the distribution of deaths (by age group) and the prevalence of PrionD and AD. One-way analysis of variance with multiple comparisons was used to compare mean ages in the Prion-only, AD-only, and Prion-AD groups.

RESULTS

Overlap of PrionD and AZ

For CERAD staging of AD, we examined multiple brain regions, including the hippocampus, entorhinal cortex, transentorhinal cortex, inferior temporal cortex, lateral frontal cortex areas 45 and 46, lateral parietal cortex areas 39 and 40, cingulate cortex area 24, and medial occipital cortex (14, 17). Of the 266 PrionD cases reviewed for the study, 46 cases (17%) showed both PrP^{Sc} and extracellular Aβ42 plaques and were designated as the Prion-AD group (Tables 1, 2). This group included 41 sCJD cases, 4 fCJD cases, and 1 GSS case. In 18 of 46 Prion-AD cases, a sufficient number of Aβ42 plaques were detected in all cerebral cortical regions and in some subcortical regions to qualify for ''definite'' AD by CERAD criteria. In the remaining 28 Prion-AD cases, the number of Aβ42 plaques was insufficient to make a diagnosis of definite AD; therefore, we designated them as ''incipient AD.'' The 220 cases with PrP^{Sc} but no detectable Aβ42 plaques were designated as ''Prion-only'' cases.

Epidemiology

In the Prion-AD group, the mean ± SD age at death was 67.2 ± 8.8 years (n = 45 available ages) (Fig. 1B; Tables 1, 2). The mean ± SD age at death in the Prion-AD group was significantly older than that in the Prion-only group (62.8 ± 10.8 years; n = 209 available ages) (Table 2) and significantly younger than that in the 127 AD-only group seen at UCSF (76.8 ± 11.1 years) (Fig. 1B; Table 2) ($p = 0.005$ and $p < 0.001$, respectively; analysis of variance controlled for multiple comparisons) .

The female-to-male ratio was calculated for each group because the AD literature indicates that women have an increased risk for AD (18, 19). Among our 127 cases of AD-only,

the female-to-male ratio was 1.35, which is consistent with the literature (Fig. 1D; Table 2B). Among 44 Prion-AD cases (the sex of 2 cases was not reported), the female-to-male ratio was 1.32, which was similar to that in AD-only cases. In contrast, Prion-only cases were predominantly male (106 men and 95 women), and the female-to-male ratio was 0.90 (Table 2B). We combined the number of women and men from the Prion-only group with the number of women and men from the Prion-AD group to test whether the predominance of men in Prion-only cases was caused by a shift of women from the Prion-only group to the Prion-AD group. The totals (120 women and 125 men) yielded a slight male predominance with a female-to-male ratio of 0.96 (Table 2B). In the Prion-AD group, more women were older than 65 years, and slightly more men than women were aged between 50 and 64 years (Fig. 1B). These data are similar to findings showing that AD is more common in older women than in men.

PrP^{Sc} and Aβ42 Plaque Distribution

In most patients, PrP^{Sc} was distributed either in all layers of the cortex or in deep layers 4 to 6. Rarely was the distribution of PrP^{Sc} found in superficial layers 1 to 3. For cases in which PrP^{Sc} was deposited in deeper cortical layers, such as an 81-year-old woman who was near the end of the age distribution for the Prion-AD group (Fig. 2A), Aβ42 plaques tended to be distributed in superficial layers 1 to 3 (Fig. 2B). In another case of a 53-year-old woman who was at the beginning of the Prion-AD age distribution, PrP^{Sc} staining was confined to layers 5 and 6 in the lateral frontal cortex (Fig. 2C) and Aβ42 plaques were located in layer 1 (Fig. 2D). In the parietal cortex of the same patient, PrP^{Sc} accumulated in all 6 cortical layers (Fig. 2E), and Aβ42-positive plaques were found in cortical layers 1 to 5 (Fig. 2F). This suggests that Aβ42 plaques are first formed in layer 1 of the cerebral cortex and subsequently progress in order from layers 2 through 6.

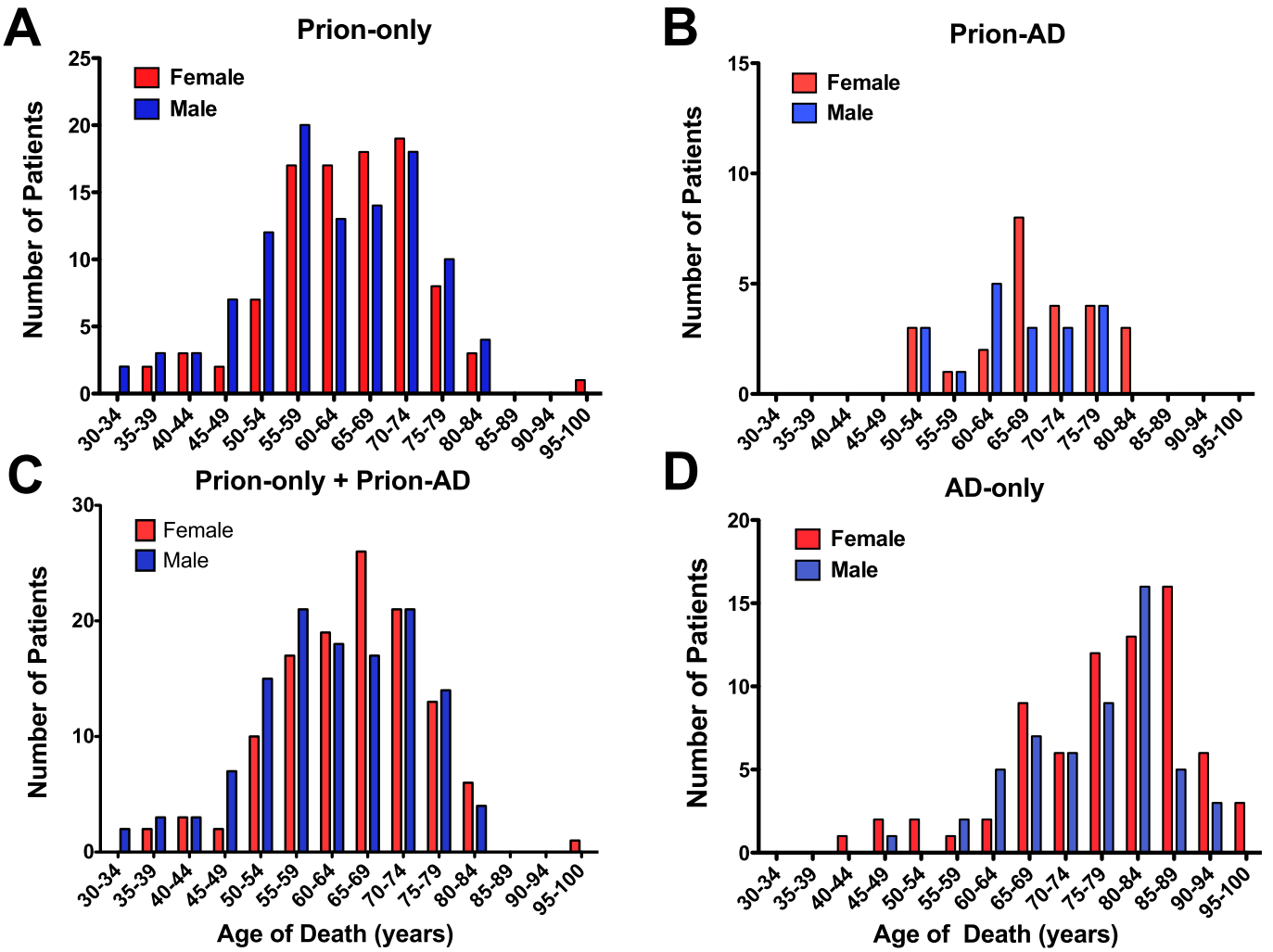


FIGURE 1. Numbers of female (red) and male (blue) patients with Prion-only, Prion-AD, and AD-only are plotted as a function of age at death. **(A)** Age at death in the Prion-only group shows a predominance of men aged 30 to 59 years, a predominance of women aged 60 to 74 years, and a predominance of men aged 75 to 84 years. **(B)** The Prion-AD group has a noticeable predominance of women, especially those aged 65 to 70 years and 80 years. **(C)** Adding the numbers of women and men in the Prion-only group to those in the Prion-AD group brings the female-to male ratio to nearly 1:1. Women predominate at ages between 60 and 69 years and between 80 and 84 years. Men tend to predominate at younger ages (from 30 to 59 years). **(D)** In the AD-only group, women predominate at almost all ages.

TABLE 1. Prion-AD Cases

Case No.	Age (years)	Sex	Diagnosis	Codon 129	PrP Mutation	Prp Type	CERAD	Brain Weight (g)
Study I: diagnosis based on Bielschowsky silver method and PrP^{Sc} and Aβ42 histoblots								
1	nd	nd	GSS	MV	Q217R	Type 1	Incipient AD	nd
2	71	M	fCJD	MV	A117V	Type 1	Definite AD	1,120
3	68	F	fCJD	MM	E200K	Type 1	Incipient AD	nd
4	62	M	fCJD	MM	R208H	Type 1	Incipient AD	nd
5	69	M	sCJD	MM	—	Type 1	Incipient AD	1,190
6	60	M	sCJD	VV	—	nd	Incipient AD	nd
7	66	F	sCJD	MV	—	Types 1 and 2	Incipient AD	1,100
8	81	F	sCJD	VV	—	Type 2	Incipient AD	1,290
9	65	F	sCJD	MM	—	Type 1	Incipient AD	nd
10	75	F	sCJD	VV	—	Type 2	Incipient AD	1,100
11	83	F	sCJD	MM	—	Type 1	Incipient AD	nd
12	73	F	sCJD	VV	—	Type 2	Incipient AD	nd
13	53	F	sCJD	nd	nd	nd	Incipient AD	nd
14	76	F	sCJD	MV	Silent 117	Type 1	Incipient AD	1,290
15	70	NA	sCJD	MM	—	nd	Definite AD	1,160
16	74	F	sCJD	MM	—	Type 1	Incipient AD	nd
17	62	F	sCJD	MM	—	Type 1	Incipient AD	1,250
18	77	F	sCJD	nd	nd	nd	Incipient AD	nd
19	73	F	sCJD	nd	nd	nd	Incipient AD	1,125
20	77	M	sCJD	MM	—	Type 1	Incipient AD	1,375
21	66	M	sCJD	nd	nd	nd	Definite AD	nd
22	60	M	sCJD	nd	nd	nd	Definite AD	nd
23	66	F	sCJD	nd	nd	nd	Incipient AD	nd
24	54	M	sCJD			Type 2	Definite AD	1,330
25	57	F	sCJD	nd	nd	nd	Incipient AD	nd
26	65	F	sCJD	MM	—	Type 1	Definite AD	nd
27	66	M	sCJD	VV	—	Type 2	Incipient AD	1,400
28	69	F	sCJD	nd	nd	nd	Incipient AD	1,175
29	73	M	sCJD	MM	—	Type 1	Definite AD	nd
30	79	F	sCJD	MM	—	Type 1	Incipient AD	1,350
31	60	M	sCJD	nd	nd	nd	Definite AD	nd
32	73	F	sCJD	MM	—	Types 1 and 2	Incipient AD	1,300
33	52	M	sCJD	VV	—	Type 2	Incipient AD	nd
34	52	F	sCJD	MM	—	Type 1	Incipient AD	1,280
35	70	M	sCJD	nd	nd	nd	Definite AD	nd
36	51	F	fCJD	MM	6ORI	Type 1	Definite AD	nd
Study II: diagnosis based on PrP^{Sc}, Aβ42, and abnormal tau immunohistochemistry								
37	66	F	sCJD	MM	—	Type 1	Definite AD	1,290
38	75	M	sCJD	VV	—	Type 2	Incipient AD	1,500
39	64	M	sCJD	MM	—	Type 1	Definite AD	1,675
40	59	M	sCJD	MM (MV)	—	Types 1 and 2	Incipient AD	1,560
41	60	F	sCJD	MM	—	Types 1 and 2	Definite AD	1,300
42	75	M	sCJD	MM	—	Type 1	Incipient AD	1,200
43	82	F	sCJD	MV	—	Type 1	Definite AD	1,250
44	66	F	sCJD	__			Incipient AD	1,290
45	79	M	sCJD	__			Definite AD	nd
46	52	M	sCJD	__			Definite AD	1,330

Alzheimer disease was classified according to CERAD criteria using the density of Aβ plaques: either definite AD (varying) or incipient AD (sparse to moderate).
F, female; M, male; MM, methionine-methionine; MV, methionine-valine; VV, valine-valine; nd, not determined.

TABLE 2. Clinical Data

	Prion-Only	Prion-AD	AD-Only	Prion-Only + Prion-AD
(A) Patient information				
Number of patients with sCJD	197	41	0	
Number of patients with fCJD*	15	4	0	
Number of patients with GSS†	6	1	0	
Total number of patients	218	46	127	
Age at death, mean ± SD, years	62.8 ± 10.8	67.2 ± 8.8	76.7 ± 11.2	
Age at onset, mean ± SD, years	59.7 ± 12.2	67.3 ± 7.8	69.7 ± 14.2	
Duration of disease, mean ± SD, months	8.7 ± 12.2	9.3 ± 10.5	87.2 ± 67.3	
Brain weights, mean ± SD, g	1,316 ± 162 (n = 49)	1,287 ± 146 (n = 23)	1,121 ± 121 (n = 15)	
(B) Number of female vs male patients				
Number of patients (female/male)	95/106	25/19	73/54	120/125
Female-to-male ratio	0.90	1.32	1.35	0.96

The number of patients in (B) differs from that in (A) because of the absence of information on the sex of some patients.
*Mutations found: E200K (7), V210I (3), R208H (1), octapeptide insertion (3), and not available (2).
†Mutations found: P102L (1), A117V (2), and Q217R (2).

Intracellular Aβ42 Peptide Accumulation in Prion-Only Cases Without Extracellular Aβ42 Plaques

To determine whether Aβ42 peptide formed and accumulated in neurons in vivo in the absence of extracellular Aβ42 plaques, we performed Aβ42 immunohistochemistry of the CA1 region of the hippocampus and the entorhinal cortex in 14 recent Prion-only cases (13 sCJD and 1 GSS). Neuron counts in the CA1 region were determined by counting neurons in 5 consecutive microscopic fields (10×). We identified 3 groups of Prion-only cases (Table 3). In the first group, Cases 1 to 5 had abundant intracellular Aβ42 in the CA1 region (Fig. 3B). The neurons of the CA1 region appeared normal in size and shape, and the mean number of neurons was 188, with only Case 1 showing a decrease in neurons to 131 (Fig. 3B; Table 3). Furthermore, Aβ42 seemed to fill a few glial cells that were identified based on their small size compared with the size of neurons (Fig. 3B). In the second group, Cases 6 to 12 showed accumulation of Aβ42 in less than 5% of neurons in the CA1 region (Fig. 3D). In some of those cells (neuron or glial), Aβ42 seemed to fill the cytoplasm, giving them a rounded appearance, although adjacent neurons appeared normal in size and contained only a small amount of Aβ42 (Fig. 3D). The mean number of neurons was 127, which verified our visual impression of nerve cell loss (Table 3). The mean number (188) of neurons found in Cases 1 to 5 and the mean number (127) of neurons found in Cases 6 to 12 were significantly different ($p = 0.01$, *t*-test). In the third group, Cases 13 and 14 had low Aβ42 levels and no nerve cell loss, showing a mean number of 212 neurons (Table 3). These data suggest the role of another factor (in addition to Aβ42 and PrP^{Sc}) in determining neuron death.

Nerve Cell Loss Correlates With Apolipoprotein E-4

A factor that is more likely to cause nerve cell death is apolipoprotein E-4 (APOE-4). The *APOE-4* allele is associated with increased risk and earlier onset of AD; *APOE-3/3* allele is associated with a significantly lower risk of AD, and *APOE-2* allele is associated with a decreased risk of AD and delayed onset of AD (20–22). Apolipoprotein E is normally expressed in astrocytes but not in neurons (23). Under conditions of stress, such as in mice treated with kainic acid, many hippocampal neurons express APOE-4. In another study, the expression of APOE-4 was associated with neuronal apoptosis (24).

We used APOE-4–specific antibodies to stain the CA1 region in 14 Prion-only cases (Table 3). The percentage of nerve cell bodies in the CA1 region containing Aβ42 and APOE-4 was quantified. Three markedly different results were obtained (Table 3). First, Cases 1 to 5 had low APOE-4 content in CA1 neurons and relatively few punctate APOE-4 deposits in the neuropil (Fig. 3E), which were associated with very little loss of CA1 neurons (Table 3). Second, Cases 6 to 12 had high intracellular neuronal APOE-4 content (Fig. 3F), which was associated with significant CA1 neuron loss and many APOE-4 deposits in the neuropil (Fig. 3F; Table 3). Third, Cases 13 and 14 contained very low amounts of APOE-4 and no associated nerve cell loss (Table 3). Accumulation of APOE-4 in neurons, resulting in apoptosis, was reflected by the large numbers of shrunken neurons with degenerating nuclei (Fig. 3F, arrows). We conclude that nerve cell loss was related to increased levels of APOE-4 and not to Aβ42.

sCJD(VV2) Induces Aβ in Hu BrnAggs

Human BrnAggs were derived from the brain of a 16-week human fetus, which did not contain extracellular Aβ42 plaques of AD. Starting from the second day in culture, BrnAggs were exposed to a normal human brain homogenate, exposed to a human sCJD(VV2) brain homogenate, or not exposed to any brain substance (None). The BrnAggs were terminated at 35 days. We pooled groups of 20 Hu BrnAggs to test each condition by Western blot analysis. An increase in Aβ42 was visibly recognizable in BrnAggs exposed to sCJD(VV2) but not in BrnAggs exposed to a normal brain homogenate or in BrnAggs not exposed to any brain substance (None) (Fig. 4A). Densitometry of Aβ bands using BioQuant Life Science software produced densities of 542,

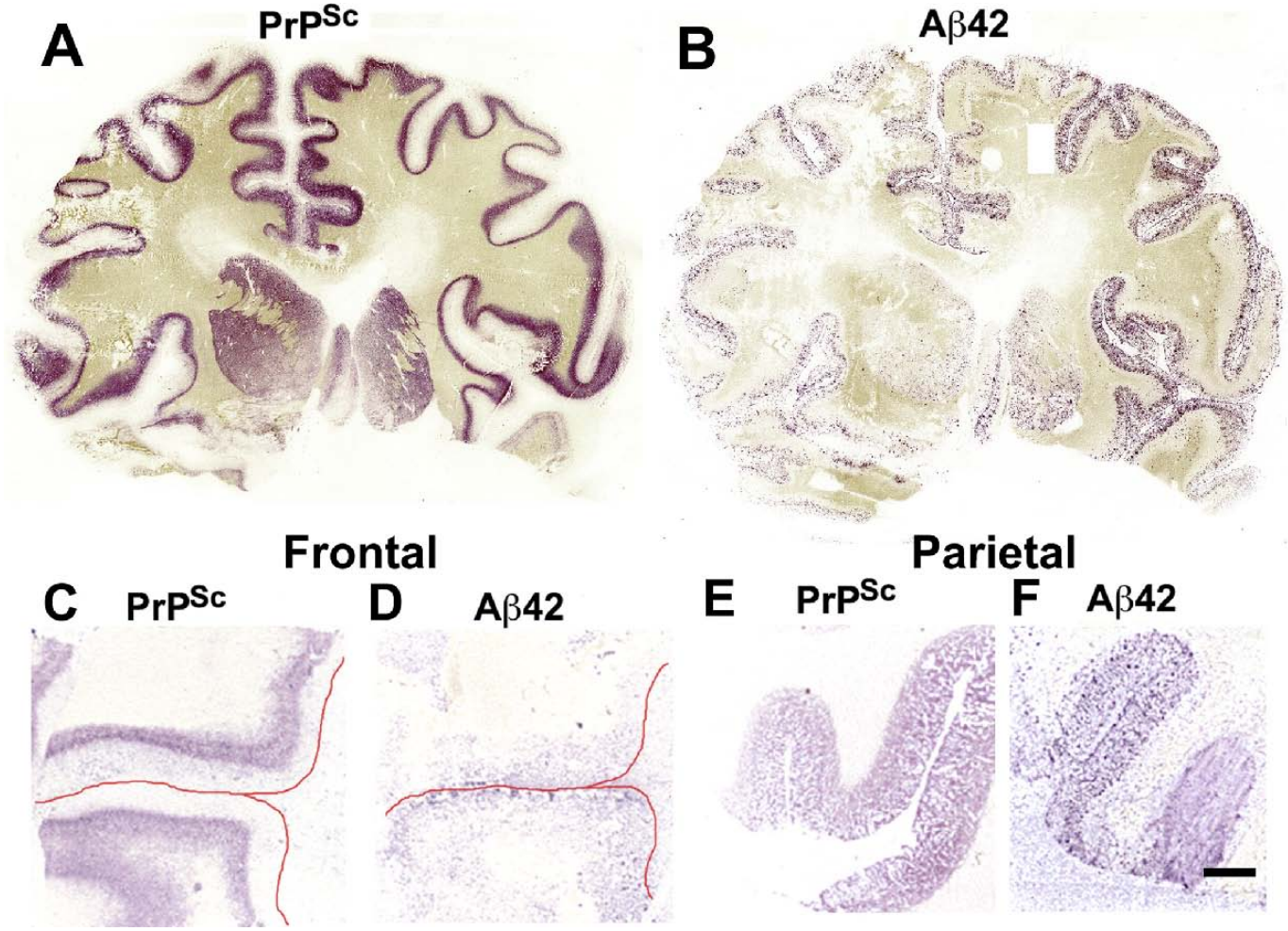


FIGURE 2. Scrapie-associated prion protein and Aβ (Aβ42) histoblot analyses of the cerebrum in sCJD. **(A, B)** Full coronal sections from an sCJD case with incipient AD (Case 8; Table 1), not strictly fulfilling the CERAD criteria for AD. 3F4 immunostaining shows that PrP^{Sc} is located mostly in cortical layers 5 and 6 **(A)**. 4G8 monoclonal antibody immunostaining indicates that Aβ42 plaques are located mostly in layers 1 to 4 of the cerebral cortex. A few Aβ42 plaques are also located in the caudate nucleus and putamen, particularly on the right **(B)**. **(C, D)** Sections of the lateral frontal cortex in another sCJD case with incipient AD (Case 13; Table 1). Scrapie-associated prion protein also localizes to cortical layers 5 and 6 **(C)**. A few Aβ42-positive plaques are located in layer 1. Red lines mark the location of the pial surface **(D)**. **(E, F)** Sections of the parietal cortex of the same case shown in **(C)** and **(D)**. Scrapie-associated prion protein **(E)** and Aβ42 plaques **(F)** are located in layers 1 to 6. Scale bars = **(C–F)** 4 mm.

1,506, and 450 for None, sCJD(VV2), and Normal Brain, respectively (Fig. 4B), indicating that sCJD(VV2) induced a 3.35-fold increase in Aβ compared with a normal brain homogenate and a 2.78-fold increase in Aβ compared with no treatment. To confirm infection with sCJD(VV2), we treated the samples with proteinase K and subjected them to Western blot analysis. Prion protein–specific antibodies yielded the 3 signature proteinase K–resistant bands in Hu BrnAggs exposed to sCJD(VV2) but none in Hu BrnAggs exposed to a normal brain homogenate or in Hu BrnAggs not exposed to brain tissue (None) (Fig. 4A).

In a preliminary study, Hu BrnAggs were exposed to sCJD(VV2) for immunohistochemical localization of Aβ42. Prion exposure was begun on Day 2 in culture and was terminated at 35 days. Human BrnAggs were fixed with 4% paraformaldehyde. Immunostaining using anti-Aβ antibodies showed that all 5 of the Hu BrnAggs exposed to sCJD(VV2) were filled with large and small Aβ42 aggregates in nerve cell bodies and in the neuropil (data were not shown because a normal age-matched human brain homogenate from a nondemented patient was not used as control). When examined by double labeling (Aβ42 and the lysosomal marker cathepsin D), Aβ42 deposits seemed to be located mainly in the cytosol and only minimally in lysosomes. In contrast, BrnAggs not exposed to prions showed little or no Aβ42. These observations suggest that sCJD prions induced intraneuronal Aβ42 peptide accumulation relatively rapidly and without formation of extracellular Aβ42 plaques.

sCJD(VV2) Induces Hτ in Hu BrnAggs

Untreated Hu BrnAggs produced large bands of tau (between 35 and 50 kDa) (Fig. 4C). Exposure to a normal brain homogenate produced smaller bands (between 35 and 45 kDa) and 2 very weak bands (~60 and >100 kDa) (Fig. 4C). Exposure to sCJD(VV2)

TABLE 3. Prion-Only Cases

	Diagnosis	Sex	Age (years)	Codon 129*	PrP^{Sc} Accumulation in the CA1 Region and Other Neuropathologies	Aβ42 (%)	APOE-4 Load	Neuron Cell Counts
1	sCJD	F	57	MM2	Proximal CA1: little, finely granular PrP^{Sc} staining Distal CA1: moderate amount of finely granular PrP^{Sc} staining	90	28.0	131
2	GSS	M	49	nd	CA4 to CA1: a background of dense, finely granular PrP^{Sc} staining surrounds neurons and GSS plaques	80	21.0	217
3	sCJD	M	60	MM2	Proximal CA1: little or no PrP^{Sc} staining Distal CA1: moderate degree of PrP^{Sc} course deposits	80	68.0	200
4	sCJD	M	68	MV2	CA4 to CA1: dense, finely granular PrP^{Sc} staining surrounding neurons	70	9.4	169
5	sCJD	M	57	MV2	Proximal CA1: very little PrP^{Sc} Distal CA1: moderate amount of finely granular PrP^{Sc} surrounding neurons	40	24.6	223
6	sCJD	M	74	MV1/MV2	CA1: dense, finely granular PrP^{Sc}; PrP plaques in SR and SO (less in SP) surround nerve cells; a few nerve cell bodies and dendrites contain intracellular PrP^{Sc}	<5	60.0	122
7	sCJD	M	Anonymous	MV1/MV2	Same as Case 6 with some exceptions: dense, finely granular PrP^{Sc} and plaque-like deposits surround dendrites; PrP^{Sc} accumulation in nerve cell bodies Distal: severe vacuolation in the CA1 region to EC	<5	nd	155
8	sCJD	F	66	VV2	All 3 cortical layers of the CA1 region stain strongly for PrP^{Sc} with focally dense, finely granular PrP^{Sc} associated with neuronal cell membranes	<5	35.4	105
9	sCJD	M	66	MV1/MV2	Proximal CA1: no PrP^{Sc} Distal: moderate PrP^{Sc} in all layers of the CA1 region	<5	55.2	113
10	sCJD	F	60	MM2	Proximal CA1: moderate amount of coarse PrP^{Sc} filling all 3 layers	<5	56.4	179
11	sCJD	F	67	MV1/MV2	CA4 to CA1: dense, finely granular PrP^{Sc} in all 3 layers	<5	40.0	115
12	sCJD	M	72	nd	CA1: sparse amount of finely granular PrP^{Sc} in all 3 layers	<5	60.0	100
13	sCJD	M	42	nd	Proximal CA1: no PrP^{Sc} Distal CA1: ~15% of nerve cell bodies are filled with PrP^{Sc}	<1	0.5	210
14	sCJD	F	56	MM1	CA4 to CA1: little, finely granular PrP^{Sc} in SR; ~50% of CA1 neurons contain intracellular PrP^{Sc}; coarsely granular PrP^{Sc} is also scattered around dendrites (sparse in SP)	<1	2.3	244

Comparison of Aβ42, APOE-4, and nerve cell loss in 5 regions (10×) of the CA1 region of the hippocampus. Apolipoprotein E-4 load is the number of APOE-4–positive neurons in the CA1 region multiplied by APOE-4 staining intensity (0, *none*; 1, *mild*; 2, *moderate*; 3, *severe*). The *t*-test value for APOE-4 load in Cases 1 to 5 and Cases 6 to 12 is 0.07.

**PRNP* genotype and PrP^{Sc} phenotype.

EC, entorhinal cortex; F, female; M, male; MM, methionine-methionine; MV, methionine-valine; nd, not determined; SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum; VV, valine-valine.

produced bands between 40 and 50 kDa and strong bands (from 60 to >100 kDa) of phosphorylated tau (Fig. 4C).

In another preliminary study, we made BrnAggs from transgenic mice expressing human mutated tau(P301L). These mouse tau(P301L) BrnAggs spontaneously form small numbers of neurons containing Hτ in nerve cell bodies and dendrites. When Mo BrnAggs were exposed to an Aβ42-containing homogenate from a transgenic ($HuAPP_{695}SWE$)2576 mouse, the number of neurons containing abnormal tau increased approximately 2-fold. In contrast, when tau(P301L) BrnAggs were exposed to a brain homogenate from a wild-type mouse infected with Rocky Mountain Laboratories scrapie prions containing PrP^{Sc}, the number of neurons and their processes containing abnormal tau increased 10-fold. These results were reproduced in triplicate. The findings argue that exposure to Aβ42 and PrP^{Sc} increases the levels of Hτ inclusions in

tau(P301L) BrnAggs. We did not use normal age-matched human brain homogenate controls in these studies, which is why we classified them as preliminary (data not shown).

Hτ in 6 Age-Matched Controls

The brains of 6 patients (aged 48–69 years and with no history of dementia) were used as controls. One control had no AT8-positive Hτ (Fig. 5A). Three controls had varying amounts of AT8 positivity consisting of small numbers of Hτ bearing neuropil threads grouped into clusters (Figs. 5B–D, arrows). Increasing amounts of Hτ-positive neuropil threads mixed with intensely stained individual nerve cell bodies occurring in clusters were seen in 2 other controls (Figs. 5E, F). All Hτ existed as small single or multiple disconnected and patchy Hτ deposits in the presubiculum, entorhinal cortex, or transentorhinal cortex.

Hτ in the MTL of PrionD and AD Cases

Unlike controls in which abnormal tau staining was focal, Hτ staining of MTL in CJD-only (19 cases), GSS-only (1 case), CJD-AD (7 cases), and AD-only (5 cases) was continuous or multifocal and extended from the entorhinal cortex into the transentorhinal cortex (Figs. 6C, F). Cases of CJD-only and CJD-AD showed strong positive and continuous staining in layers 1 and 2 of the entorhinal cortex and transentorhinal cortex (Fig. 6B). Lower-intensity Hτ staining was also present in neuropil threads and neurons in deeper layers of the entorhinal cortex in CJD-only cases. Cases of CJD-AD showed intense immunostaining in the superficial layers and less intense immunostaining in the deeper layers of the entorhinal cortex (Figs. 6E, F). In AD-only cases, many Hτ threads and nerve cell bodies were stained (Figs. 6H, I). The most intense staining occurred in the GSS-only case (Figs. 6K, L). In all of the groups, the presubiculum showed little Hτ staining. The hippocampal CA1 region and subiculum were intensely stained in CJD-AD and AD-only cases, whereas the GSS-only case had staining in portions of the CA1 region and the subiculum.

Hτ Quantification

The density and distribution of AT8-immunopositive Hτ were estimated in the LC/RN of the pons and in the MTL in 36 cases (Fig. 7). These cases included 20 Prion-only (19 sCJD and 1 GSS), 7 CJD-AD, 5 AD-only, and 4 ''other'' (3 cases of TDP-43 encephalopathy and 1 case of dementia lacking distinctive pathology) cases. We compared Hτ loads by estimating the intensity of Hτ staining multiplied by the area stained in the LC/RN and MTL. The highest densities of Hτ were found in the MTL, and the lowest densities of Hτ were found in the LC/RN (Figs. 7A, B). The densities of Hτ in the MTL were significantly higher in the AD-only and CJD-AD groups compared with the Prion-only group (*t*-test, $p < 0.001$; Fig. 7B). In Prion-only cases, a score of 1 or 2 represents the presence of any focal Hτ staining in the entorhinal cortex; therefore, it may represent nonspecific Hτ staining. A score of 3 or more represents a continuous deposition of disease-related Hτ in the entorhinal cortex and transentorhinal cortex (Fig. 6C). The sole score of 48 occurred in the GSS case (Fig. 7A).

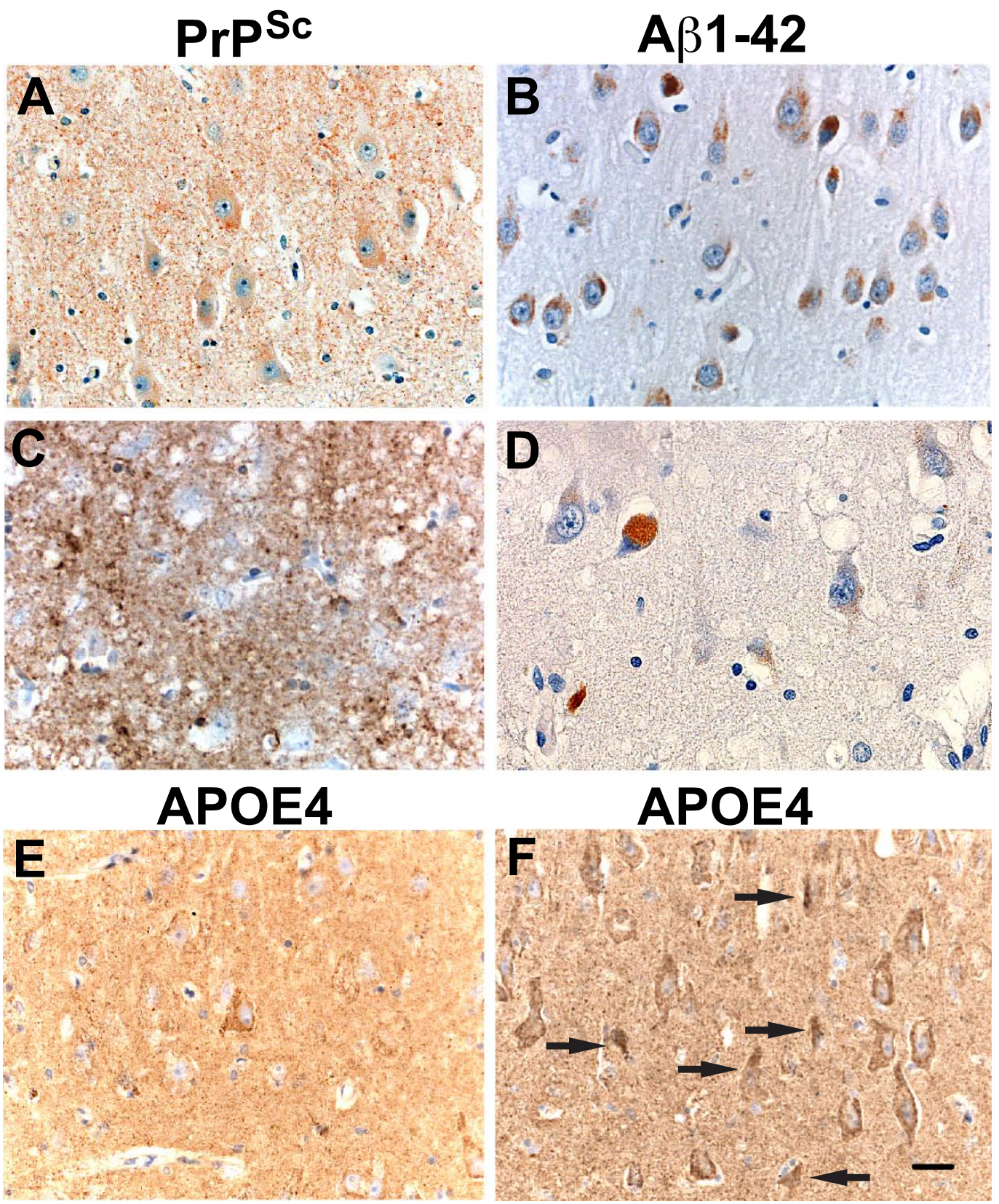
DISCUSSION

In examining 266 cases of PrionD, we found that 46 patients (17%) also had AD and AD-like changes. In a previous study, Hainfellner et al (1) looked at 110 cases of CJD using anti-PrP, anti-Aβ, and anti-tau immunostaining plus the Bielschowsky silver method to examine a single neocortical block of brain tissue. They found a 10.9% overlap of PrionD and AD and proposed that AD and prion pathology are coincidental events (1). Like Hainfellner et al (1), we also used anti-PrP, anti-Aβ, and anti-tau immunostaining plus the Bielschowsky silver method, but we examined multiple neocortical areas.

The yearly incidence of sCJD, which accounts for approximately 90% of PrionD cases, is 1 to 2 cases per million (25). The incidence of PrionD is similar to its prevalence because patients usually die within 1 year of diagnosis. The yearly incidence of AD may be as high as approximately 150,000 cases per million (26), but patients may live 10 years or longer; thus, the prevalence is much greater than the incidence. We could not find adequate data on the prevalence of AD by age to allow us to determine whether our findings were coincidental or mechanistic, but the proportion of Prion-AD cases found in both studies strongly suggests that the large size (25%–30%) of the preclinical and definite AD populations overlaps with many populations, including the population of PrionD cases. Because we found large quantities of Aβ inside neurons in Prion-AD cases, our results are consistent with the concepts that the mechanism of AD in PrionD starts with PrP^{Sc} stimulation of Aβ synthesis and that its accumulation inside neurons is not relevant to the proportion of preclinical and definite AD. This hypothesis is supported by several factors linking PrionD and AD.

The age of patients in the Prion-AD group led us to think that PrionD might induce AD or AD-like changes. In our series, the findings of concomitant Prion-AD changes began abruptly at about age 50 years and seemed to end just as abruptly at age 84 years, except for 1 outlier who died at age 93 years. The older age (84 years) of Prion-AD cases coincides with the end of the Prion-only group. By contrast, Aβ-associated pathology in AD-only cases continued to increase in the brain until the last AD case died at the age of approximately 100 years (7, 27). The implication of these data is that AD does not induce PrionD, whereas PrionD induces AD and AD-like pathology. These observations led us to look further to see whether we could identify mechanisms by which PrionD could cause or accelerate the onset of AD in susceptible patients.

Other factors that link PrionD to AD are the formation and neuronal accumulation of PrP^{Sc}, which increases levels of Aβ (28), and the subsequent formation and accumulation of APOE-4 in neurons (Fig. 3; Table 3). Both events also occur in AD-only cases without PrP^{Sc} stimulation. We saw low levels of PrP^{Sc}, high intraneuronal levels of Aβ, and low levels of intraneuronal APOE-4 in patients with PrionD who died without apparent nerve cell loss in the CA1 region of the hippocampus. We saw high levels of PrP^{Sc}, low levels of intracellular Aβ, and the transition of APOE-4 to high levels in patients who demonstrated nerve cell loss (Table 3). The study by Umeda et al (29) supports the role of Aβ oligomers in causing nerve cell death through an endoplasmic reticulum stress mechanism;



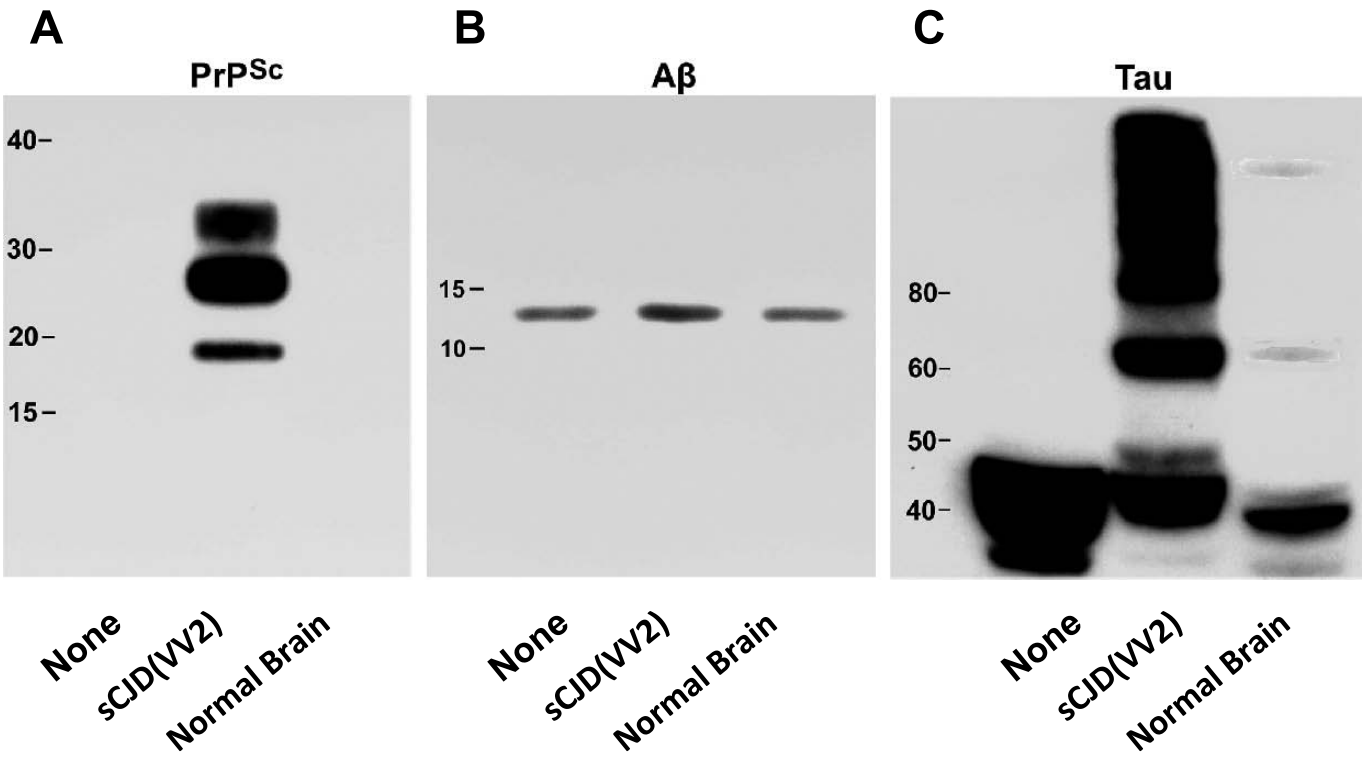


FIGURE 4. Western immunoblots stained for PrP^{Sc}, Aβ (Aβ42), and tau protein in 20 pooled Hu BrnAggs. Each set of BrnAggs was not treated (None), treated with a human brain homogenate from a patient with sCJD (sCJD(VV2)), or treated with a normal human brain homogenate (Normal Brain). **(A)** All 3 of the treatments were exposed to proteinase K to show protease-resistant PrP^{Sc}. **(B)** Antibodies to Aβ peptide showed that exposure to sCJD(VV2) resulted in increased levels of Aβ in BrnAggs, whereas no treatment (None) and treatment with human brain homogenate (Normal Brain) did not. **(C)** Antibodies to tau protein showed that sCJD(VV2) was the only treatment that caused a large amount of phosphorylated tau (molecular mass >60 kDa) to be generated. The normal brain homogenate generated very small bands (60 and >100 kDa). Molecular weights are indicated in each immunoblot.

however, they did not mention APOE-4 activation, a factor that most likely links Aβ pathology to apoptosis of nerve cells (24). Apolipoprotein E-4 binds tightly to Aβ42 to clear it from neurons (30). As a result, soluble oligomeric Aβ levels are increased by APOE-4 (31). We found that concomitant changes in PrionD and AD occurred in patients aged between 50 and 85 years—the time when APOE-4 (a known risk factor for AD) (32, 33) and neprilysin-α (34) (the predominant degrading enzyme of Aβ40 and Aβ42) (35) exert their maximal effects on the development of AD (36).

Apolipoprotein E alleles are not a risk factor for PrionD (37), but APOE-4 contributes to nerve cell death in patients with PrionD. In our Prion-only cases, Cases 1 to 5 had low PrP^{Sc} levels, high intraneuronal levels of Aβ42, low numbers of neurons expressing APOE-4, and little neuronal loss in the CA1 region (Table 3). Prion-only cases (Cases 6–12) that had high PrP^{Sc} levels, low Aβ42 levels, and high APOE-4 levels had a 40% mean loss of CA1 neurons. In Cases 6 to 12, we also saw punctate APOE-4 immunostaining in the neuropil between nerve cell bodies—findings that were not observed in Cases 1 to 5. We believe that the neuropil APOE-4 resulted from nerve cell death, releasing APOE-4 into extracellular space. Neuronal death was an apoptotic event based on the number of shrunken neurons with fragmented nuclei containing APOE-4 seen in the section (Fig. 3F). Therefore, it seems that increasing PrP^{Sc} levels triggers an increase in APOE-4.

FIGURE 3. Intracellular Aβ (Aβ42) peptide and APOE-4 accumulation in neurons in sCJD cases: 2 Prion-only autopsy cases. **(A)** Scrapie-associated prion protein staining of Case 1 (Table 3) shows finely granular deposits of PrP^{Sc} in the neuropil and nerve cell bodies of the CA1 region. **(B)** Ninety percent of CA1 neurons contain Aβ42 cytoplasmic inclusions and 3 glial cells with intracellular Aβ42. **(C)** Scrapie-associated prion protein immunohistochemistry in Case 7 (Table 3) shows dense, coarse, and plaque-like PrP^{Sc} deposits filling the entire neuropil, surrounding neurons, and infiltrating neurons. **(D)** Intracytoplasmic inclusions of Aβ42 are strongly positive in a few cells that could be glial or neuronal. Three adjacent neurons contain very light brown peroxidase staining indicating Aβ42 positivity; it is admittedly difficult to differentiate Aβ42 immunoreactivity inclusions from lipofuscin. There are also fewer neurons in the field compared with **(B)**. **(E)** The same case (Case 1) as in **(A)** shows a single neuron at the center of the field that is APOE-4–immunopositive. There are a few scatted granular deposits of APOE-4 in the neuropil. **(F)** The same case (Case 7) as in **(C)** shows that almost all neurons in this field contain APOE-4. In addition, there are many punctate APOE-4 deposits in the neuropil, possibly resulting from degeneration of APOE-4–positive neurons. Arrows indicate apoptotic neurons. Scale bar = **(F)** 30 μm (applies to all panels).

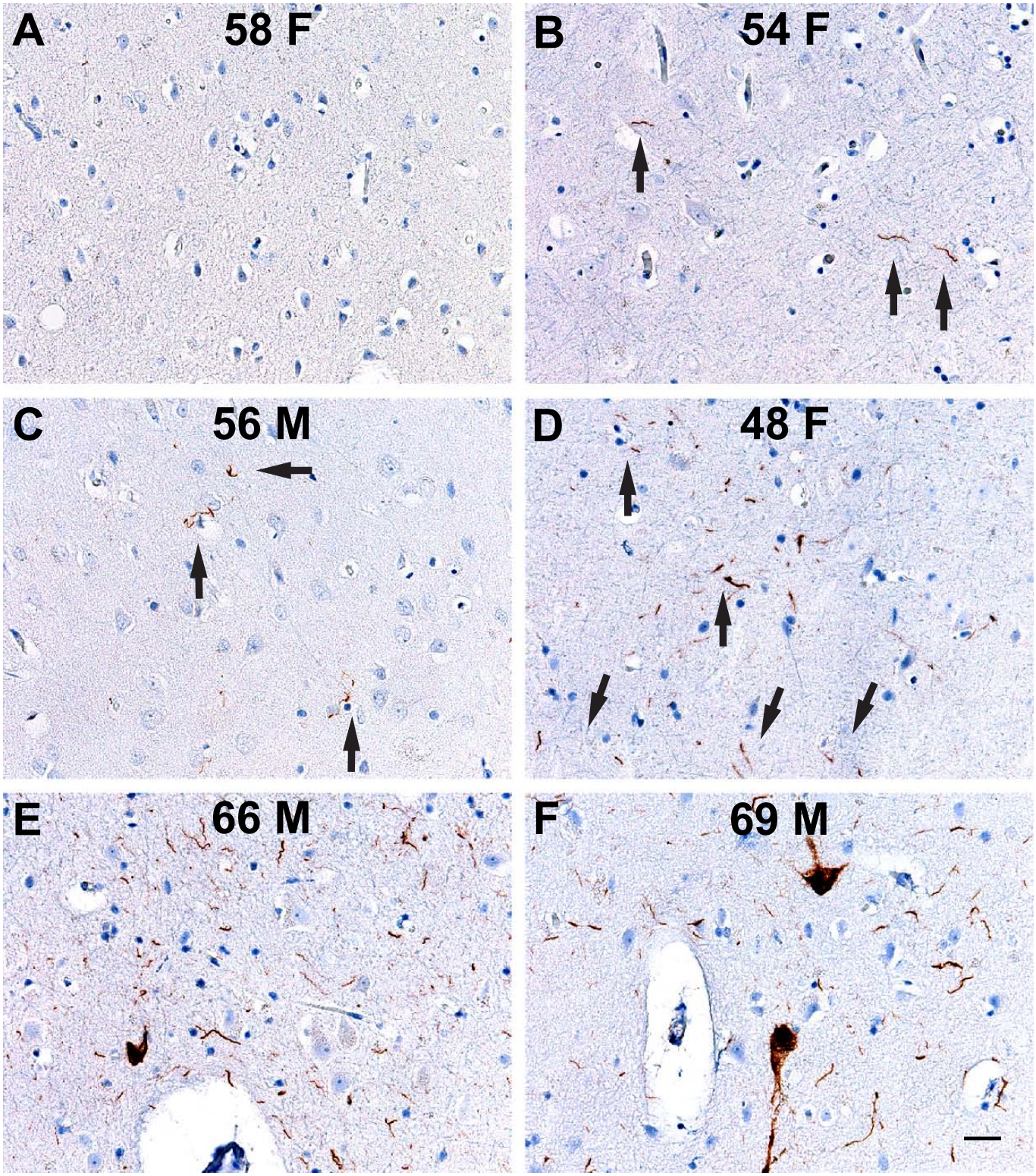


FIGURE 5. No Aβ (Aβ42) was found in the MTL of 6 age-matched control cases without dementia or neurologic disease that was stained for Aβ42 and Hτ. Abnormal tau was found in single or several clusters in the entorhinal cortex. The age (in years) and sex (F, female; M, male) of each control case are indicated. **(A)** No tau is found. **(B–D)** One to 3 microscopic foci of tau-positive neuropil threads are found. Arrows point to some of the neuropil threads. **(E, F)** Larger clusters of neuropil threads and occasional cell bodies contain abnormal tau. Clusters were isolated and not connected. Scale bar = **(F)** 50 μm (applies to all panels).

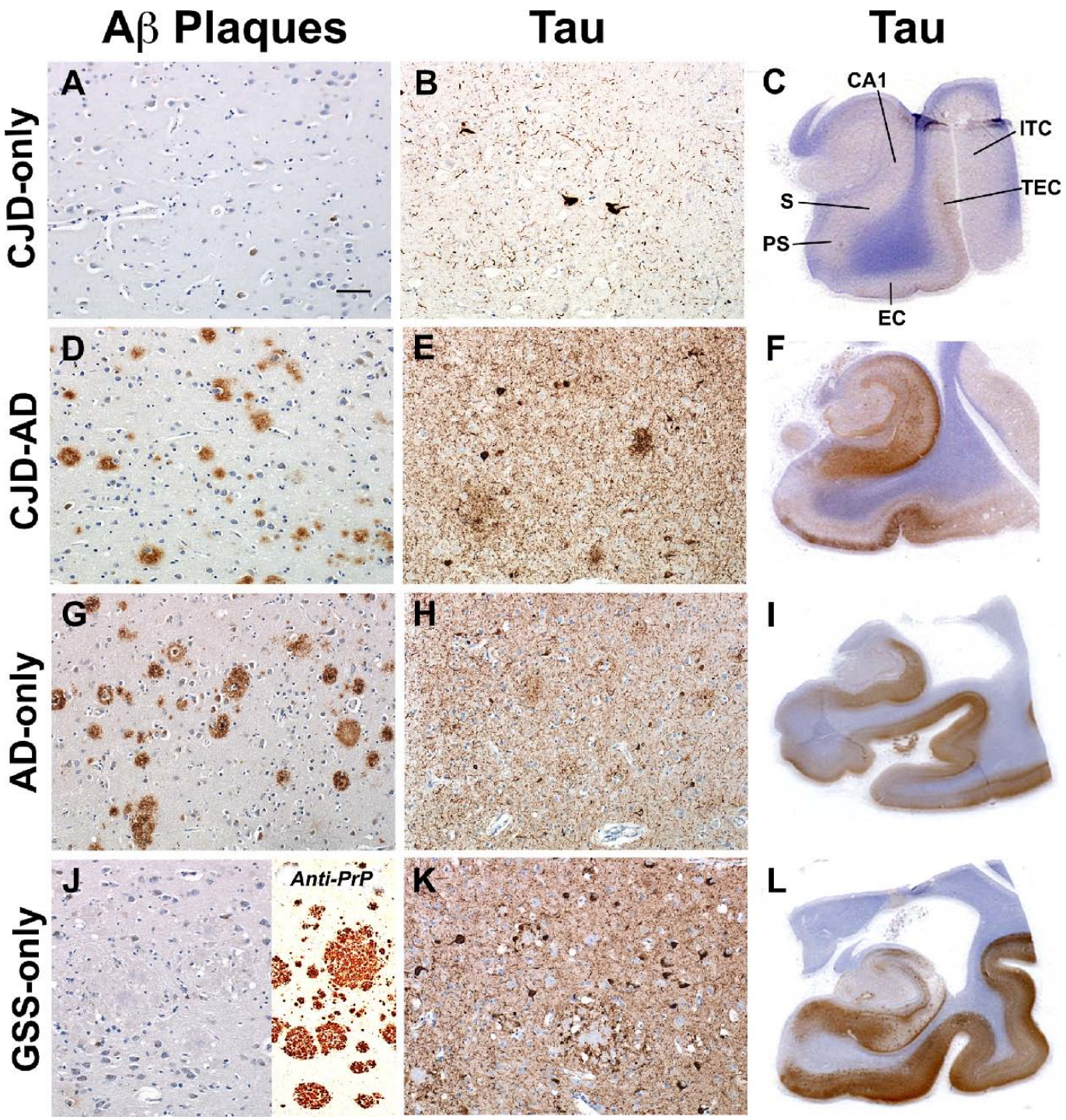


FIGURE 6. The number and distribution of Aβ plaques and Hτ-positive threads and nerve cell bodies in the MTL differ from those of controls in Prion-only cases **(A–C)**, Prion-AD (CJD-AD) cases **(D–F)**, and AD-only cases **(G–I)**. A single GSS-only case showed the most intense staining of neuropil threads and neuronal cell bodies and no Aβ plaques **(J–L)**. Gerstmann-Sträussler-Scheinker disease plaques were identified with the 3F4 antibody (**J**; inset). β-Amyloid plaques were immunostained with 4G8 monoclonal antibody (left), and Hτ was immunostained with AT8 (middle and right). Microscopic sections in the 2 left columns were photographed with a 20× objective lens of a microscope. Tau-immunopositive cell bodies and neuropil threads were present in the entorhinal cortex in all groups of cases (the least in Prion-only cases and the most in the GSS-only case) **(B, E, H, K)**. **(C, F, I, L)** Whole mounts of the MTL are stained for Hτ **(C, F, I, L)**. EC, entorhinal cortex; ITC, inferior temporal cortex; PS, presubiculum; S, subiculum; TEC, transentorhinal cortex. Scale bar = **(A)** 100 μm (applies to other micrographs in the left and middle columns).

A **Abnormal Tau Scores**

Prion-only		Prion-AD		AD-only		Other	
LC/RN	MTL	LC/RN	MTL	LC/RN	MTL	LC/RN	MTL
3	1	5	32	9	32	0	0
3	4	1	28	3	40	3	3
1	5	2	14	8	48	1	1
1	1	3	20	6	40	4	2
2	8	4	48	4	10.5	2.0 ± 1.8	1.5 ± 1.3
1	8	1	12	5.5 ± 2.6	34.0 ± 5.7		
2	8.5	2	9				
1	1	2.6 ± 1.5	23.2 ± 14				
1	4						
2	4						
1	15						
1	8						
2	2.5						
1	2						
1	4						
1	1						
4	GSS 48						
2	4						
3	3						
6	8						
2.0 ± 1.3	7.0 ± 10.3						

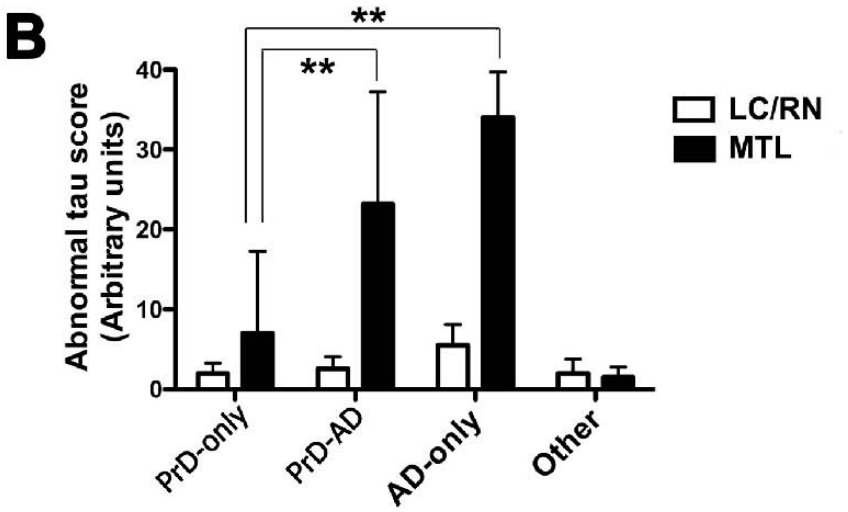


FIGURE 7. Quantification of Hτ immunostaining in the LC/RN and MTL of autopsy subjects, 4 of which were shown in Figure 6. **(A)** The estimated amounts of Hτ-containing neuropil threads, nerve cell bodies, and dystrophic neurites were measured by densitometry in 20 Prion-only (PrD-only), 7 Prion-AD (PrD-AD), 5 AD-only, and 4 ''other'' (3 cases of TDP-43 encephalopathy and 1 case of dementia lacking distinctive abnormalities) cases. Values for each category are presented as mean ± SD. **(B)** Abnormal tau scores are shown as a function of each disease group (mean ± SD). When MTL values were compared, abnormal tau was significantly higher in the AD-only and Prion-AD groups than in the Prion-only group (19 CJD and 1 GSS marked in the figure) (** $p < 0.001$, *t*-test).

The sole exception to this pattern was Case 2 (Table 3), a case of GSS associated with a 9-octapeptide repeat insertion in the *PRNP* gene. This patient had a 10-year history of tremors that progressed to a severe mental disorder during the last 2 years of life; 80% of his CA1 neurons contained Aβ42 peptide, and PrP^{Sc} deposits were densely granular and associated with GSS plaques. Nerve cell loss was not identified in the CA1 region, although APOE-4 levels were not particularly low (Table 3).

The literature indicates that homozygosity at codon 129 of the *PRNP* gene (either M/M or V/V) is associated with an increased risk of developing sCJD, iatrogenic CJD, or variant CJD (38–40). In the German population, M/M homozygosity at codon 129 is a risk factor for early-onset AD, but not for late-onset AD (41). In the Dutch population, the risk of developing early-onset AD is higher for V/V homozygotes than for M/M homozygotes (42). In our study, an overrepresentation of codon 129 homozygosity (84%) was found in the Prion-only and Prion-AD groups compared with the general US population (49%) (Table 3). However, the PrionD-AD association was not exclusively related to homozygosity because a small percentage of Prion-AD cases in our cohort were heterozygous (M/V).

Our results suggest that being female is a risk factor for the simultaneous occurrence of PrionD and AD. It has been reported that women have a 2-fold higher risk of developing AD than men. This increased risk has been attributed to the loss of estrogen after menopause (18, 19, 43) and to the fact that women tend to live longer than men (44). In our Prion-AD

cases, the female-to-male ratio was 1.32, which was similar to that in the AD-only group (1.35) but different from that in the Prion-only group (0.90). Because nearly all of our Prion-only cases died within less than 1 year of disease onset, the longevity of women was not an issue in Prion-AD cases. In contrast, the sex distribution associated with intracellular Aβ42 accumulation in the absence of Aβ42 plaques in our Prion-only cases was predominantly male (9 of 14 cases) (Table 3). This observation suggests that the emergence of amyloid plaques of AD has a greater association with women. It is interesting that 3 of 4 MV Prion-AD cases were female (Table 1).

The second classic neuropathologic feature of AD is accumulation of Hτ. Several clinical studies have identified increased levels of total tau and phosphorylated tau in the cerebrospinal fluid of patients with CJD (45–47). Our autopsy study seems to be the first to demonstrate increased amounts of abnormal tau (Hτ) and Aβ42 peptides on immunohistochemistry and biochemistry in Prion-only, Prion-AD, and AD-only cases versus ''other'' neurodegenerative disease cases, including TDP-43 encephalopathy and dementia lacking distinct histology (Fig. 7). The highest levels of Hτ were present in the AD-only group, with progressively less Hτ in the Prion-AD and Prion-only groups. In the Prion-only group (Fig. 7), 4 tau loads in the MTL were given a tau score of 1, which did not represent continuous tau immunostaining in the entorhinal cortex and transentorhinal cortex but represented Hτ staining that was similar to our 6 controls. The ''other'' group of neurodegenerative disease had the least Hτ score from 0 to 3.

In summary, in some patients, PrionD induces pathology consistent with AD (increased number of extracellular Aβ42 plaques, increased intracellular Aβ42 peptide accumulation, and increased deposition of Hτ). Prion disease is able to induce AD changes but AD does not seem to induce PrionD. Thus, we propose that PrionD is a disorder of 4 aberrant protein conformers (i.e., PrP^{Sc}, Aβ42, APOE-4, and Hτ) and that each of these proteins contributes to the pathogenesis of PrionD.

Does it matter whether PrionD induces or accelerates AD? It certainly did not matter to the patient who died of PrionD within a year. A better understanding of the combined effects of PrP^{Sc}, Aβ42, APOE-4, and Hτ on PrionD, however, may provide potential targets for prion therapy. More importantly, understanding how PrionD can incite AD-like changes may lead to the discovery of additional triggers and clues to control the growing epidemic of AD.

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