

## Neuronal Expression and Intracellular Localization of Presenilins in Normal and Alzheimer Disease Brains

DUONG P. HUYNH, HARRY V. VINTERS, DIANE H. D. HO, VINH V. HO, AND STEFAN-M. PULST

**Abstract.** The expression patterns of presenilin 1 (PS1) and presenilin 2 (PS2) in human normal and Alzheimer disease (AD) brains were investigated using antibodies to specific N-terminal peptides of PS1 (Alzh14A and Alzh14B) and PS2 (Alzh1A-AB). The antibodies to peptides Alzh14A (Alzh14A-AB) and Alzh14B (Alzh14B-AB) detected the full-length protein (~63 kDa) and the N-terminal-processed fragment (36 kDa) of PS1, while the Alzh1A-AB detected mainly the N-terminal-processed fragment (36 kDa) of PS2. Immunofluorescent staining detected by confocal microscopy suggested that both native PS1 and PS2 are localized mainly in the Golgi/ER apparatus. Immunohistochemical studies of human temporal lobes from 2 normal and 5 sporadic Alzheimer brains revealed high levels of PS1 and PS2 expression in the granule cell layer and pyramidal neurons of the hippocampus. Strong immunoreactivity was found in reactive astrocytes and neurofibrillary tangles of all 5 Alzheimer brains. In contrast, only 2 sporadic Alzheimer brains showed presenilin-positive neuritic plaques. These observations suggest that presenilins may be involved in the pathology of some cases of sporadic AD.

**Key Words:** Familiar Alzheimer disease; Hippocampus; Human brain; Neocortex; Presenilin 1; Presenilin 2.

### INTRODUCTION

Early-onset familial Alzheimer disease (EOFAD) is a neurodegenerative disorder caused by defect in at least 3 genes. The first EOFAD gene identified was the *amyloid  $\beta$ -protein precursor (APP)* gene on chromosome 21 (1). Mutations of this gene account for only 5% of the affected Alzheimer families. The other two genes, *presenilin 1 (PS1)* and *presenilin 2 (PS2)*, were recently identified (2, 3) and account for about 70% of all EOFAD. The *Presenilin 1* gene is located on chromosome 14 and the *PS2* gene on chromosome 1. Both presenilins are highly homologous, with 67% identity, and show high homology to 2 *Caenorhabditis elegans* gene products, *sel-12* (4, 5) and *spe-4* (6), which have been suggested to be involved in receptor localization and cytoplasmic protein redistribution.

Since its identification, 25 different missense mutations in the PS1 gene have been found in 52 EOFAD families (3, 7, 8–13). Two different missense mutations have been found in 7 Volga-German families and in an Italian AD family (2, 14). Most of these mutations are distributed throughout the presenilins; however, the second transmembrane domain (TM2) and the sixth hydrophilic loop have the highest number of mutations, suggesting that

these two regions may confer specific biological functions (9).

Both the *PS1* and *PS2* genes encode proteins with distinct primary structures. Presenilin 1 has 467 amino acid residues with a predicted molecular weight (MW) of 53 kDa. The PS2 protein has 448 amino acid residues with a predicted MW of 50 kDa. The putative secondary structures of both presenilins are similar, with at least 7 to 10 transmembrane domains. Both presenilins share an overall identity of 67%, with the highest similarity observed in the putative TM domains (2, 3). Alternatively spliced isoforms of presenilins have been found in brain, fibroblasts, and leukocytes. One shorter isoform results from alternative splicing of exon 8 (aa residues 257–290 in HL-VI region) (14). In the N-terminal region, alternative splicing results in the deletion of the VRSQ sequence (codons 26 to 29) (7, 15). The VRSQ sequence is the potential phosphorylation site for protein kinase C and casein kinase H, which suggests that the N-terminal domain is located intracellularly. Therefore, the presence of the VRSQ sequence and the absence of a membrane signal peptide suggests that presenilins likely localize to the intracellular compartment.

The patterns of expression of both presenilins appear to be similar and widespread as shown by Northern blotting, in situ hybridization and immunocytochemistry. By Northern blot analysis, presenilin mRNAs were found in the heart, brain, placenta, lung, skeletal muscle, and the pancreas (3, 14). In the human brain, presenilin mRNAs were detected in the amygdala, caudate, corpus callosum, hippocampus, hypothalamus, substantia nigra, subthalamic nuclei, and thalamic nuclei (3, 14). Using in situ hybridization with antisense oligonucleotides to PS1 and PS2 cDNAs, widespread expression of presenilin mRNAs was found in mouse brain, with the highest expression found in the hippocampal formation, the entorhinal cortex, and the cerebellum (16, 17). Using an

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From the Neurogenetics Laboratory, Burns and Allen Research Institute, Cedars-Sinai Medical Center, Room D1056, 8700 Beverly Boulevard, Los Angeles, CA 90048 (DPH, DHDH, VVH, SMP) and the Department of Pathology and Laboratory Medicine, UCLA School of Medicine (HVH), Los Angeles, California.

Correspondence to: Stefan-M. Pulst, Neurogenetics Laboratory, Burns and Allen Research Institute, Cedars-Sinai Medical Center, Room D1056, 8700 Beverly Boulevard, Los Angeles, CA 90048.

This work was supported in part by the Carmen and Louis Warschaw Endowment Fund for Neurology (SMP), the Ruth and Lawrence Harvey Endowment Fund for Neuroscience (SMP), and grant P30 AG 10123 from NIH (HVH).

antibody to PS1, we found that presenilin 1 was widely expressed in adult mouse brain, with high levels found in pyramidal neurons and sensory neurons (18). Furthermore, fusion proteins containing presenilins expressed in a human glioma cell line showed that presenilins were localized in the endoplasmic reticulum and Golgi bodies (16). Other studies have also found that the full-length *PS1* and *PS2* are processed into smaller fragments of 32–35 kDa for the N-terminal fragments and approximately 17 kDa for the C-terminal fragments (19, 20). The similarity of the primary amino acid structure and pattern of mRNA expression of presenilin mRNA suggests that both presenilins may have similar biological functions. However, a mutation in either presenilin is needed to cause EOFAD-linked AD pathology, suggesting that both presenilins have functions that are linked to a single pathway.

Here we report the localization of both PS1 and PS2 in normal and AD neocortex and hippocampus using specific antibodies to both presenilins. In normal human tissues, the highest levels of expression of presenilins were found in pyramidal neurons. In AD tissues, both presenilins were found in neuritic plaques (NP) and neurofibrillary tangles (NFT).

## METHODS

### Antibody Production

We raised rabbit antibodies (ABs) against C-terminal and N-terminal peptides. For presenilin 1 (3), we selected peptide Alzh14A, which is located at aa 55–68 (PQGMSRQVVE-QDEE), and peptide Alzh14B, which is located at aa residues 331–344 (NDDGGFSEEWEAQR). For presenilin 2 (2), peptide Alzh1A is located at aa residues 30–43 (SCQEGRQGPEDGEN). Peptides Alzh14A and Alzh1A were from the N-terminal domain, and peptide Alzh14B was from the 6th hydrophilic loop. Each peptide was conjugated to keyhole limpet hemocyanin and injected into 2 rabbits. Collected antisera were used directly for immunofluorescent staining or were affinity purified for Western blots and immunohistochemistry as previously described (21).

### Western Blots and Protein Extraction

Proteins from human and mouse brain tissues and cell lines were extracted according to Wood et al (22). Frozen tissues were thawed in lysis buffer A (100 mM Hepes, pH 7.4, 0.4% Sucrose, 1 mM Pefabloc, 1 mM EGTA, 1 µg/ml Peptastain A, 2 µg/ml Aprotinin, 50 µg/ml Leupeptin) and homogenized using a polytron homogenizer. The protein extracts were first spun at 1000 g (3100 rpm in JA17 rotor) for 5 minutes (min). The P1 pellet containing crude nuclei and cell debris was washed once with lysis buffer and resuspended in buffer B (buffer A plus 1% Triton X-100). The supernatant (S1) was spun at 10,000 g (10,000 rpm in JA17) for 30 min. The pellet (P2) containing mitochondria and synaptosomes was washed once and resuspended in buffer B. The supernatant (S2) was centrifuged at 105,000 g (54,000 rpm in TLN100 rotor) for 1 hour (h). Again the pellet (P3) containing Golgi apparatus,

endoplasmic reticulum, and plasma membrane was washed once with buffer A and resuspended in buffer B. The supernatant (S3) was aliquoted and stored at  $-80^{\circ}\text{C}$ . Protein concentrations were determined by the BioRad DC-Bradford Protein Assay Kit. Prior to loading onto the polyacrylamide gel, proteins were concentrated using a Microcon 10 (Amicon). Fifty to 100 µg of protein was loaded per lane in a BIORAD pre-made 4 to 20% gradient SDS-polyacrylamide mini-gel and resolved at 100 V for 1 to 2 h. Fractionated proteins were transferred to a nitrocellulose filter (23). The filter was rinsed briefly with TBS (150 mM NaCl, 50 mM Tris, pH 8.0), blocked with 5% nonfat dried milk, and then incubated with the desired dilution of tested antibodies overnight at  $4^{\circ}\text{C}$ . The primary antibody was detected using the BioRad Immunoblot Assay Kit for alkaline phosphatase-conjugated anti-Rabbit IgG.

### Immunohistochemistry

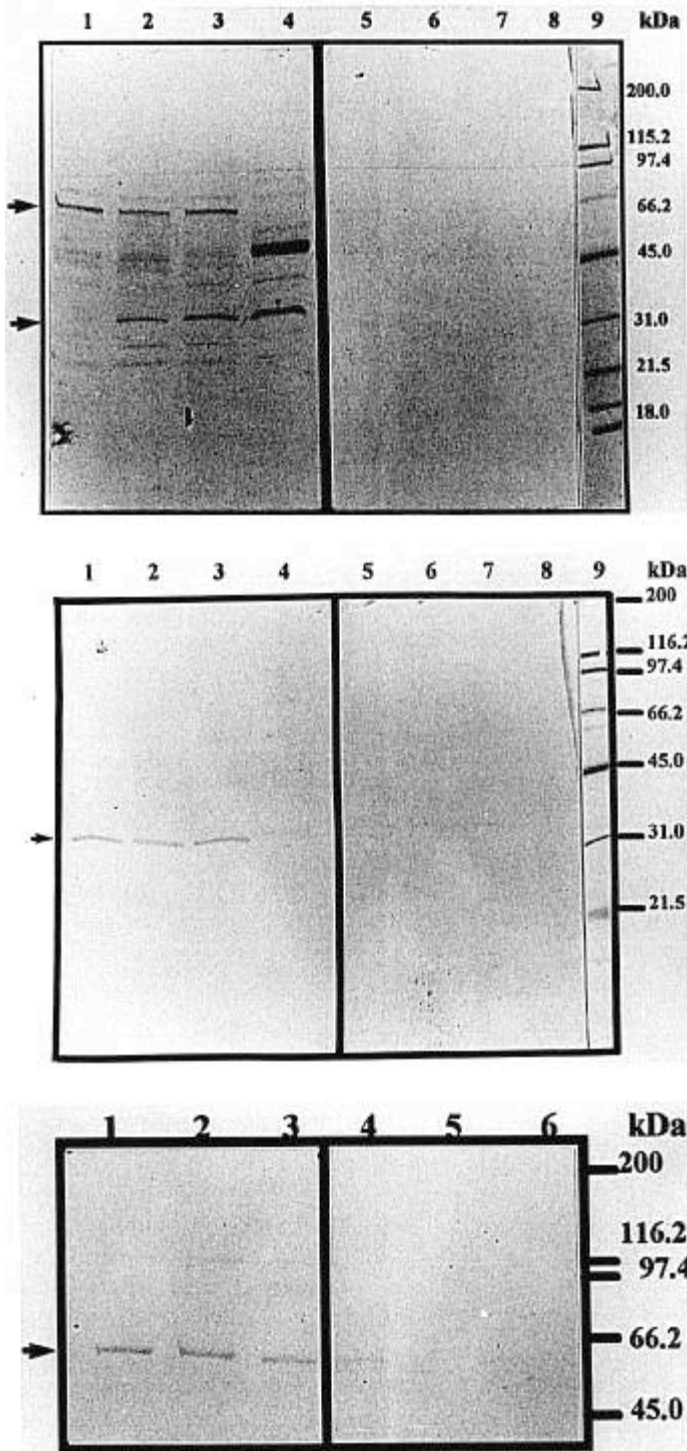
Control and sporadic Alzheimer neocortex and hippocampal formation blocks were formalin fixed and embedded in paraffin. Seven-micron sections were cut and mounted onto Superplus microscopic slides (Fisher Scientific). The sections were rehydrated by rinsing twice at 5 min interval in xylene, 100% ethanol, 95% ethanol, and 70% ethanol. Prior to antibody treatment, rehydrated sections were treated as previously described (21). Sections were incubated with 20 µg/ml of affinity-purified presenilin antibodies overnight at  $4^{\circ}\text{C}$ . Primary antibodies were detected using a Vector ABC elite Peroxidase kit (Vector, CA), enhanced by DAB enhancer, and visualized with diaminobenzidine (DAB) (Biomed, CA). Sections were counterstained with aqueous hematoxylin (Xy-med, CA). Controls consisted of antibodies preabsorbed with 100 µM of the respective peptide and preimmune sera at comparable concentrations (1/500).

### Cell Culture

SK-N-M (HTB10, ATTC) is a neuronal cell line derived from human neuroblastoma. SK-N-M cells were grown in T75 tissue flasks containing MEM + 10% FBS +  $1\times$  antimycotic agent for 72 h at  $37^{\circ}\text{C}$ , stripped using 0.5% Trypsin/1mM EDTA, washed with culture media, and distributed in 5,000 to 10,000 cells per well in 4-well slides for morphological studies.

### Immunofluorescence

The following antibodies were used: 1:100 dilution of rabbit anti-presenilin antibodies Alzh14A-AB, Alzh14B-AB, and Alzh1A-AB. In double-labeling experiments, mouse monoclonal antibodies to Golgi proteins,  $\beta$ GOP (Sigma) and 58K8 (Sigma) were co-incubated with presenilin antibodies. Cells were grown in 4-well culture slides for 36 h at  $37^{\circ}\text{C}$  and 95%  $\text{O}_2$ . Cells were washed gently 2 times with cold DPBS and fixed for 5 min with a 1:1 mixture of cold methanol and acetone. Cells were then washed again and preincubated with an antibody Dilution Solution (3% protease-free BSA in DPBS) for 30 min at room temperature. Cells were incubated for 1 h at room temperature with 1/100 dilution of presenilin antisera and with 1/40 dilution of a mouse monoclonal antibody to  $\beta$ GOP or 58K8. Cells were washed gently 3 times with cold DPBS and were incubated for another hour at room temperature with



**Fig. 1.** A. Immunoblots of protein extracts from human brains from normal individuals (lanes 1 and 5), patients with Alzheimer disease (lanes 2-3 and 6-7), and mouse brain (lanes 4 and 8). Lanes 1-4 were incubated with Alzh14A-AB, and lanes 5-8 were incubated with preabsorbed Alzh14A-AB. Panel B, lanes 1-4, were incubated with Alzh1A-AB, and lanes 5-8 were incubated with peptide-absorbed Alzh1A-AB. Alzh1A-AB did not detect the mouse PS2. Lane 9 in each panel shows Broad Range MW standards from BIORAD. Panel C shows immunoblots of protein extracts from normal (lanes 1 and 4)

1/200 dilution of TRITC-conjugated, goat affinity-purified anti-Rabbit IgG (Sigma, T6778) and FITC-conjugated goat anti-mouse IgG. Cells were washed 4 times with cold DPBS and mounted. Fluorescence microscopy was achieved using a Zeiss LSM 210 confocal microscope equipped with FITC and Rhodamine filters. Photographs were obtained using a Sony Video printer UP-5200MD.

To determine potential cross-reactivity of secondary antibodies, cells were incubated with either rabbit presenilin antibody or mouse 58K8 and  $\beta$ GOP antibodies followed by either FITC-conjugated anti-rabbit IgG or TRITC-conjugated anti-Mouse IgG. We saw no FITC fluorescence in cells incubated with mouse primary antibodies or TRITC fluorescence in cells incubated with primary rabbit presenilin antibody, indicating that the secondary antibodies did not cross-react.

**RESULTS**

**Biochemical Analyses of Presenilins 1 and 2**

For biochemical and immunohistochemical analyses of PS1 and PS2, we generated rabbit polyclonal antisera to different peptides of PS1 and PS2. Peptide Alzh14A and Alzh14B are located at residues 55-68 and 331-344 of PS1 (3), respectively. Peptide Alzh1A is located at residues 30-43 of PS2 (2). We chose PS1 and PS2 peptides in such a way that they did not have significant homologies to each other, other regions of PS1 or PS2, or to known proteins.

To establish the specificity of the antibodies to presenilins, we performed Western blot analyses of protein extracts of normal, Alzheimer human neocortex, and mouse brain. Figures 1A and B show a Western blot of normal, Alzheimer neocortex, and mouse brain with affinity-purified antibodies to Alzh14A and Alzh1A peptides. When protein samples were incubated with 1x Laemmli buffer at 37°C water bath for 12 to 24 h, the affinity-purified Alzh14A antibody (AB) detected bands approximately at 63 kDa and 36 kDa in human brains (Fig. 1A, lanes 1-3). A 53 kDa and a weak 36 kDa band were detected in mouse brain (Fig. 1A, lane 4). Although the amount of protein was identical in all samples, the level of the 36 kDa protein was lower in the control than the AD brain. However, when the samples were boiled for 7 min, 2 bands at 36 and ~125 kDa were detected by the Alzh14A-AB (Not shown). All bands were absorbed out when Alzh14A-AB was preincubated with peptide Alzh14A (Fig. 1A, lanes 5-8). The affinity-purified Alzh14B-AB also detected a strong band at ~63 kDa (Fig. 1C, lanes 1-3). This band was not detected by Alzh14B-AB preabsorbed with its peptide (Fig. 1C, lanes 4-6). The Alzh14B-AB did not

← and Alzheimer (lanes 2, 3, 5, and 6) human brains with Alzh14B-AB (lanes 1-3) and with Alzh14B-AB preabsorbed with its peptide (lanes 4-6).

detect the 17–20 kDa C-terminal fragment shown by other studies (19, 20).

Similar observations were made with an antibody to peptide Alzh1A (Alzh1A-AB) of presenilin 2. When protein samples were incubated with 1× Laemmli's buffer at 37°C overnight, the Alzh1A-AB detected a single band at 36 kDa that is close to the N-terminal fragment of PS2 (19) (Fig. 1B, lanes 1–3). The 36 kDa protein was barely detectable by the Alzh1A-AB preabsorbed with peptide Alzh1A (Fig. 1B, lanes 5–8). However, when the samples were boiled for 7 min, 2 bands at 36 and ~63 kDa were detected (not shown). The mouse PS2 was faintly detected by the Alzh1A-AB (Fig. 1B, lane 4).

#### Immunocytochemical Localization of Presenilin 1 and 2 in SK-M-N Cells

We used immunofluorescence analysis of the human neuroblastoma SK-M-N(ATCC HTB10) cell line to determine the cellular localization of presenilins. As shown in Figure 2, antibodies to both PS1 (Fig. 2A) and PS2 (Fig. 2G) gave similar punctated staining patterns in the cytoplasmic and the perinuclear regions of SK-M-N cells. For comparison, SK-M-N cells were double labeled with PS1 or PS2 antibodies and a mouse monoclonal antibody to a Golgi protein, 58K8 (Fig. 2B, H), and analyzed by laser confocal fluorescent microscopy. When the staining of either presenilin 1 or 2 was superimposed with the 58K8 staining, colocalization of presenilin to the Golgi apparatus was observed (Fig. 2C, J). Rabbit preimmune sera gave no significant background staining (Fig. 2E, I).

#### Distribution of Presenilins 1 and 2 in Normal Brain

To determine the cellular distribution of presenilins in human brain, we stained 2 normal and 5 sporadic Alzheimer brains of matched ages using the PS1 antibody Alzh14A-AB and the PS2 antibody Alzh1A-AB. In the normal hippocampal formation, pyramidal neurons (Fig. 3E) in the CA1-CA4 regions stained stronger than the granule neurons in the dentate gyrus (Fig. 3C). The Alzh14A-AB preabsorbed with 100 μM of peptide 14A showed no visible staining (Fig. 3A). In the normal neocortex, pyramidal neurons were also more immunoreactive than other neuronal populations with the Alzh14A-AB (data not shown).

The distribution of presenilin 2 in human neocortex and hippocampal formation was similar to that of PS1 and was localized mainly in the gray matter (Fig. 3). The PS2 antibody (Alzh1A-AB) strongly stained pyramidal neurons in both the neocortex and hippocampal formation (Fig. 3F). Granular neurons of the cortical layers and the dentate gyrus were weakly stained (Fig. 3D). The Alzh1A-AB preabsorbed with 100 μM of peptide Alzh1A showed no detectable staining (Fig. 3B). Cells

in the cortical molecular layer were weakly stained by both presenilin antibodies (Fig. 4A, C).

#### Distribution of Presenilins 1 and 2 in the Brains of AD Patients

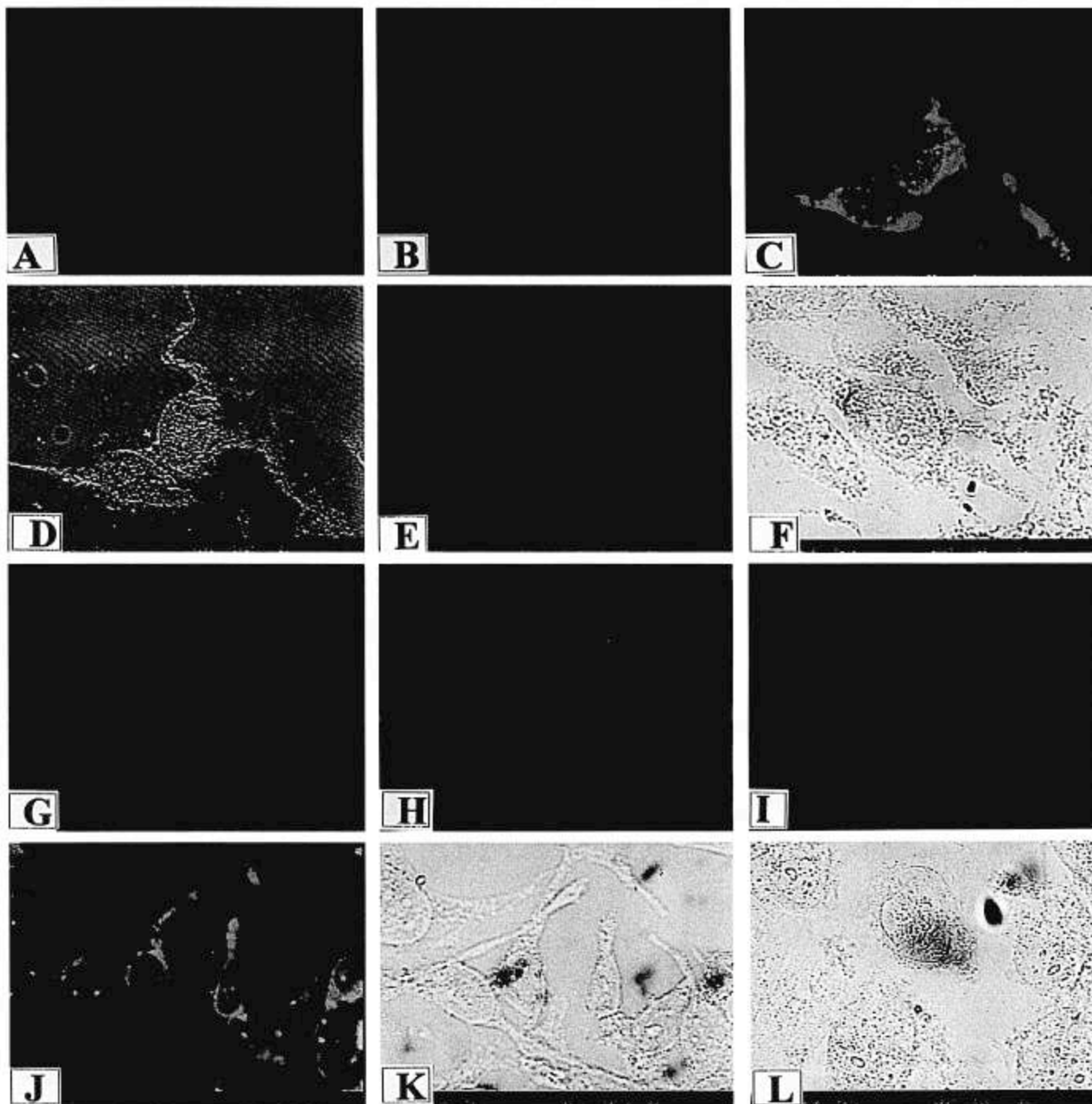
In AD brains, both PS1 and PS2 antibodies strongly stained neurofibrillary tangles, reactive astrocytes, and neuritic plaques (Figs. 4, 5). In the neocortex and hippocampus, reactive astrocytes in the molecular layers (Fig. 4B, D) and neurofibrillary tangles (Fig. 5E, F) were strongly stained by both Alzh14A-AB and Alzh1A-AB in all 5 AD cases. In contrast, neuritic plaques in only 2 of the 5 AD brains were stained by presenilin antibodies (Fig. 5A–D). Interestingly, PS1 staining of NPs (Fig. 5A–C) was significantly weaker than PS2 staining (Fig. 5B–D). Furthermore, the overall intracellular staining pattern in the AD brain appeared more discontinuous and clumped (Fig. 5), unlike the staining pattern observed in the control brains (Fig. 3). Both PS1 and PS2 antibodies preabsorbed with their respective peptides failed to detect presenilins in AD brains (not shown).

#### DISCUSSION

Information about the distribution patterns of presenilins in CNS is essential for investigating the native function of these proteins and their roles in the pathophysiology of Alzheimer disease. To elucidate the function of presenilins 1 and 2, we produced antibodies to different peptides of the presenilins and used these antibodies to investigate the expression pattern of presenilins in the neocortex and hippocampal formation of normal human and AD brain sections.

#### Protein Processing

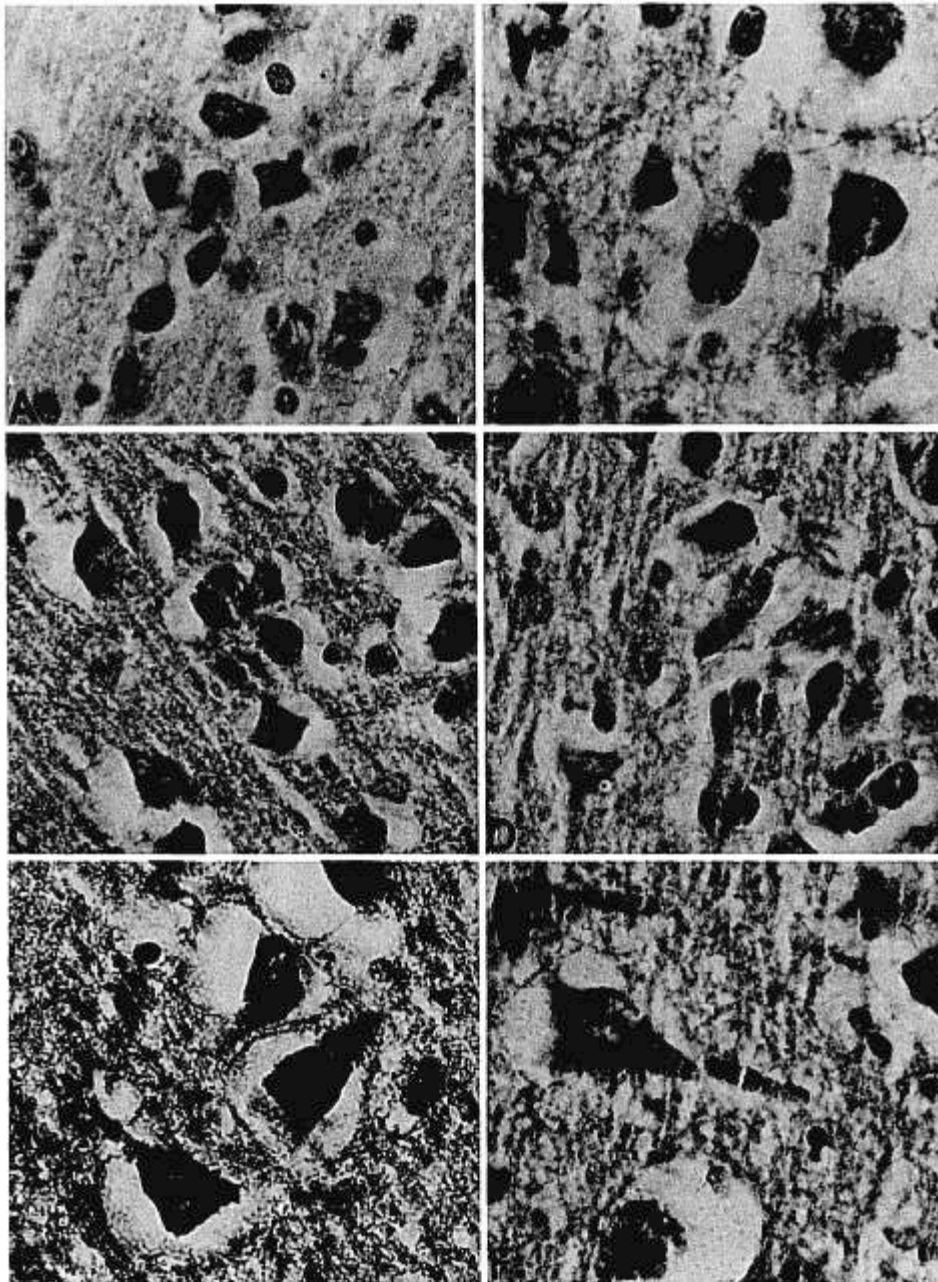
In Western blot analyses, the N-terminal peptide antibodies specifically detected at least 2 bands of approximately 63 kDa and 36 kDa for PS1 and a 36 kDa band for PS2 (Fig. 1). These proteins were detected in protein extracts from a human neuroblastoma cell line, as well as in normal and AD human cortex. These bands were specific, since preabsorbed antibodies (Fig. 1A, B, lanes 5–8) and preimmune sera did not detect them (data not shown). The ~63 kDa protein likely corresponds to the full-length PS1 protein, since antibody to a second peptide, Alzh14B-AB, also detected a protein of identical molecular weight (Fig. 1C). A similar molecular weight for the putative full-length presenilin was observed by Murphy et al (24) in cultured CHO cell protein extract. In the same study, however, Murphy et al also detected a 45 kDa protein expressed by a human recombinant presenilin gene. The 45 kDa protein was consistent with other studies that



**Fig. 2.** Immunofluorescence of SK-M-N cells with antibodies to PS1 and PS2. Cells were stained with 1/100 dilution of Alzh14A-AB (A) and 1/40 dilution of 58K8 (B), or with 1/100 dilution of Alzh1A-AB (G) and 1/40 dilution of 58K8 (H). Panels C and J are a composite of Alzh14A-AB or Alzh1A-AB with 58K8 staining, respectively. Yellow color indicates overlapping of red (for rhodamine) and green (for FITC) color. Panels E and I were stained with preimmune sera of Alzh14-AB and Alzh1A-AB, respectively. D, F, K, and L are transmitted light micrographs of panels C, E, J, and I, respectively. Bar equals 10  $\mu$ m.

used antibodies to detect recombinant expressed presenilin (14, 17, 19, 20, 25), suggesting that recombinant expressed and endogenous presenilin may migrate differently in polyacrylamide gels. Although this evi-

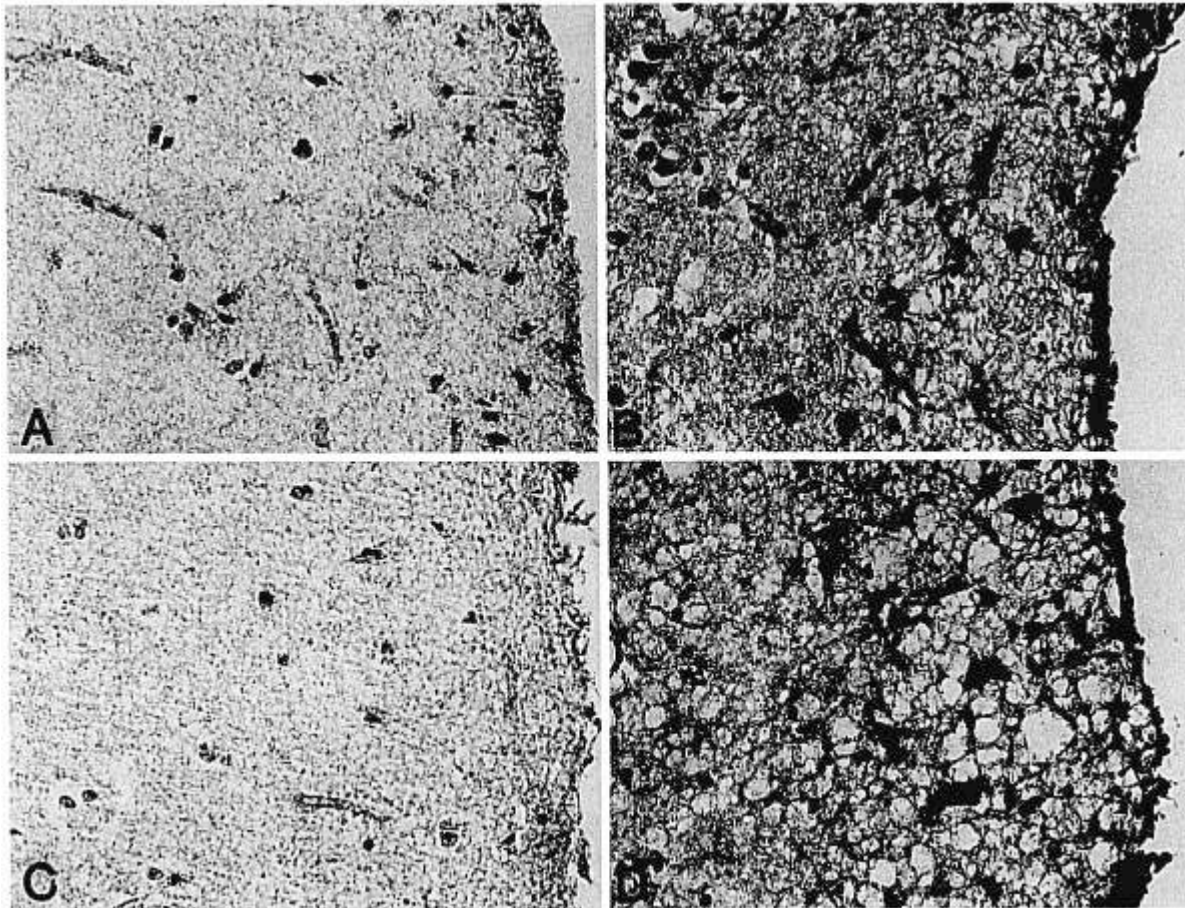
dence appears to suggest that the 63 kDa is the full-length presenilin 1, we cannot exclude the possibility that it may also represent an aggregated complex of the processed presenilin fragments.



**Fig. 3.** Localization of presenilin 1 and 2 in normal human hippocampal sections. A. Granule neurons in dentate gyrus stained with preabsorbed Alzh14A-AB. B. An adjacent section to (A) stained with preabsorbed Alzh1A-AB. C. Granule neurons of dentate gyrus stained with 20 ug/ml Alzh14A-AB. D. An adjacent section to (C) stained with Alzh1A-AB. E. Pyramidal neurons in the CA3 area stained with Alzh14A-AB. F. An adjacent section of E stained with Alzh1A-AB. Magnification, 330 $\times$ .

The 53 kDa protein observed in mouse brain is closer to the 52 kDa MW predicted from the mouse PS1 primary amino acid sequence. A protein of similar MW was observed by Citron et al (19), Kovacs et al (14), and Weidemann et al (25). This observation suggests that mouse and human endogenous PS1 may undergo different post-translational modifications.

The 36 kDa bands observed in PS1 and PS2 likely represent the N-terminal proteolytic processed fragments that have been described as a 27–33 kDa protein in brains of mouse, human, and primate (19, 22). The 125 kDa protein for PS1 and the ~63 kDa for PS2 found in boiled protein samples were likely the aggregated forms of the full-length or processed fragments



**Fig. 4.** Increased expression of PS1 and PS2 in activated astrocytes in the molecular layer of patients with AD (B and D) compared with normal controls (A and C). Sections A and B were stained with Alzh14A-AB, sections C and D with Alzh1A-AB. D. Note the intense staining of activated astrocytes (black arrow). Magnification, 330 $\times$ .

of PS1 and PS2, respectively, (Fig. 1A). A large  $\sim$ 100 kDa protein was also observed by other investigators using antibodies to different epitopes (19, 25).

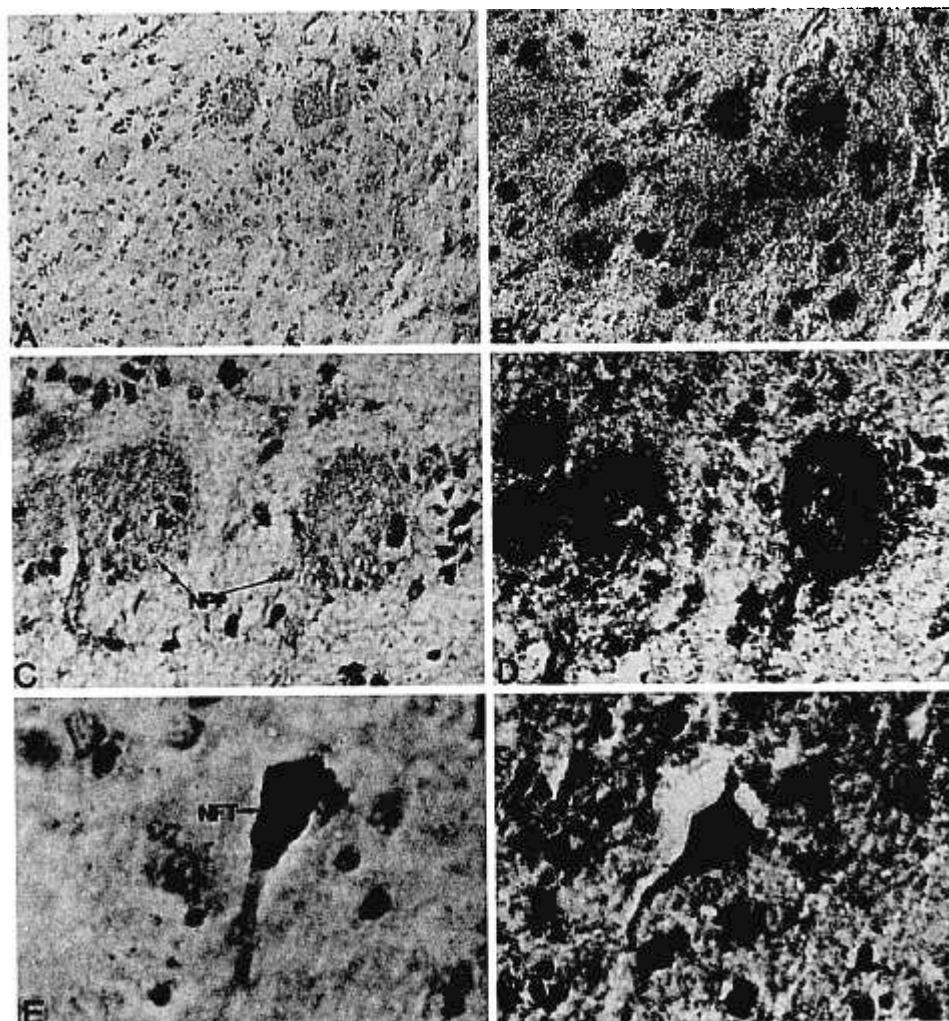
#### Intracellular Localization

Immunofluorescent studies (Fig. 2) showed that both PS1 and PS2 were intracellularly localized to the endoplasmic reticulum (ER) and Golgi apparatus. These observations are consistent with observations by Kovacs et al (14), who showed localization of FLAG-PS1 and FLAG-PS2 in the ER/Golgi complex. Interestingly, the Golgi apparatus of a subset of neurons without NFT have been shown to be fragmented and atrophic in Alzheimer disease (26). Taken together, these data are consistent with the absence of a signal peptide in the primary amino acid sequence and suggest that presenilins may play an essential role in regulating intracellular transport or post-translational modification of newly synthesized proteins. Further support for this theory is suggested by the observation that presenilins are related to the *SPE4* and *SEL-12* proteins in *Caenorhabditis elegans* that are involved in partitioning or trafficking of proteins

(3, 5). Alternatively, presenilins may be involved in maintaining the structural integrity or function of the Golgi apparatus.

#### Expression of Presenilins in Human Neocortex and Hippocampal Formation

In normal human neocortex and hippocampus, presenilin immunoreactivity was detected strongly in pyramidal neurons and weakly in granule neurons and glial cells (Figs. 3, 4). This pattern of expression was also observed for PS1 in the mouse hippocampus, where high levels of presenilin 1 were found in pyramidal neurons of CA2–CA4, and lower levels were found in granule neurons of the dentate gyrus and in the mouse neocortex (18). The different staining pattern in mouse and human neocortex may reflect species-specific protein expression. The observation of high levels of presenilin in pyramidal neurons was consistent with *in situ* hybridization studies that detected high levels of presenilin mRNAs in pyramidal neurons of the CA field and low levels in the granule neurons of the dentate gyrus (14,



**Fig. 5.** Presence of PS1 and PS2 in neurofibrillary tangles and neuritic plaques. Panels A, C, and E were stained by Alzh14A-AB. Panels B, D, and F were stained by Alzh1A-AB. A and B are low-magnification views (25 $\times$ ) of neuritic plaques (NP) in the layer II of a sporadic Alzheimer neocortex. Panels C and D are high magnification views (330 $\times$ ) of A and B, respectively. Panels E and F show neurofibrillary tangles (330 $\times$ ).

17). Lower levels of presenilin mRNA were also seen in normal glial cells in one study (17).

In the AD sections, both presenilins were detected in reactive astrocytes and in selected NFTs of all 5 AD sections, while only 2 out of 5 sporadic AD sections contained presenilin-positive neuritic plaques (Fig. 5). These staining patterns were specific based on 3 criteria: (a) negative staining with antibodies preabsorbed with their corresponding peptides; (b) no staining with preimmune sera at identical IgG concentration, and (c) different patterns of staining were seen with antibodies to presenilin 1 and 2 (Figs. 3–5).

The finding of presenilins in NFTs agree with previous studies by Murphy et al (24), who found that selected NFTs stained with both PS1 and PS2 antibodies. In contrast, our detection of presenilins in neuritic plaques was consistent with only one study (27), but not with Murphy

et al (24). In both studies, however, presenilins were not detected in reactive astrocytes. The varying observations of presenilin staining patterns in AD brains by these groups may reflect different sensitivities of antibodies to different epitopes or differences in the immunohistochemical methods used. It should be noted that both Wisniewski et al (27) and we used peptide epitopes at opposite ends of presenilin 1, while Murphy et al (24) used a peptide at the middle of presenilin 1.

Although we cannot exclude the possibility that the differential staining of plaques in sporadic AD patients could be due to subtle differences in tissue processing, it may point to heterogeneity of events leading to plaque formation in sporadic AD. Interestingly, plaque staining was stronger with the antibody to PS2 than with the PS1 antibody, although both ABs gave approximately equal staining intensity of cells in normal



human sections and in reactive astrocytes and NFTs in AD brains (Figs. 3, 5). Further studies need to determine whether the stoichiometric ratios of PS1 and PS2 are different in plaques or whether differential staining of plaque presenilins is related to differential accessibility of epitopes. The presence of presenilins in neuritic plaques in sporadic AD patients points to the importance of these proteins in sporadic AD even when they do not contain a mutation, as in patients with some forms of inherited FAD.

Since all mutations found to date in both PS1 and PS2 genes are missense mutations (3, 7–14), it has been suggested that mutation of presenilins results in a gain of function. Our observations of increasing presenilin immunoreactivity in reactive astrocytes and NFT in sporadic AD brain sections indirectly support the above hypothesis. The increased levels of wild-type presenilins may function in a dose-dependent mechanism potentially similar to a “gain of function” attributed to mutated presenilins by stimulating the synthesis of  $\beta$ -A42 (28). It remains to be determined whether mutations in other AD genes result in increased synthesis of presenilins or whether increased dosage of wild-type presenilins may cause an increase in the rate of abnormal APP fragmentation.

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Received March 20, 1997

Revision received June 9, 1997

Accepted June 11, 1997