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Presenilin-1 expression in Pick's disease

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Abstract Recent studies have reported that neuronal populations expressing low levels of presenilin-1 (PS-1) display increased vulnerability in late-onset sporadic Alzheimer's disease (AD). To examine whether this phenomenon also occurs in other neurodegenerative diseases, we performed a quantitative immunocytochemical study of PS-1 distribution in the cerebral cortex of Pick's disease (PiD) cases and non-demented individuals. In PiD cases, the percentage of PS-1-containing, Pick body (PB)-free neurons was significantly elevated only in cortical areas showing neuronal loss. In these areas, PS-1 levels, measured by immunoblotting, were often higher in PiD compared to non-demented cases. Moreover, PS-1 immunoreactivity was significantly reduced in PB-containing neurons. These data suggest that as previously shown in AD, low cellular expression of PS-1 may be associated with increased neuronal loss and cellular degeneration.

Key words Alzheimer's disease · Cerebral cortex · Neuronal loss · Pick's disease · Presenilin-1

Introduction

Germline mutations in the presenilin-1 (PS-1) gene are the most common genetic factor underlying the development of early-onset familial Alzheimer's disease (AD) (for reviews see [10, 11]). Although the role of this gene in the development of pathological changes in AD remains poorly defined, several lines of evidence suggest that PS-1 may be involved in the regulation of cell death in AD [8, 16, 22]. We have previously reported that neuronal populations that express low levels of PS-1 display increased vulnerability in the course of the degenerative process, suggesting that PS-1 may have a neuroprotective role in late-onset sporadic AD [7]. However, it has not been determined whether the preservation of PS-1-immunoreactive neurons is a specific finding in AD or if it also occurs in other neurodegenerative diseases. To address this issue, we performed a quantitative study of PS-1 distribution in the cerebral cortex of Pick's disease (PiD) cases and non-demented (ND) individuals.

Materials and methods

A total of 32 patients (20 women, 74.6 ± 1.2 years old; 12 men, 71.0 ± 1.5 years old), who died and were autopsied in the Hospitals of the University of Geneva School of Medicine, were included in the present study. Among them, 16 patients (11 women, 72.3 ± 1.8 years old; 5 men, 70.5 ± 1.0 years old) had preserved cognitive functions [7]. These patients form the ND group of the present study. The clinical diagnosis was confirmed neuropathologically by the absence of significant histopathological changes. The remaining 16 patients (9 women, 75.5 ± 2.5 years old; 7 men, 72.0 ± 2.2 years old) showed severe cognitive deterioration and major behavioral disturbances including loss of social and personal awareness, disinhibition, impulsivity, hyperorality and stereotyped behavior [13]. All of these cases had marked fronto-temporal cortical atrophy, and were characterized neuropathologically by severe ventricular enlargement, widespread gliosis, neuron loss and numerous Pick bodies (PB) in the frontal and temporal neocortex,

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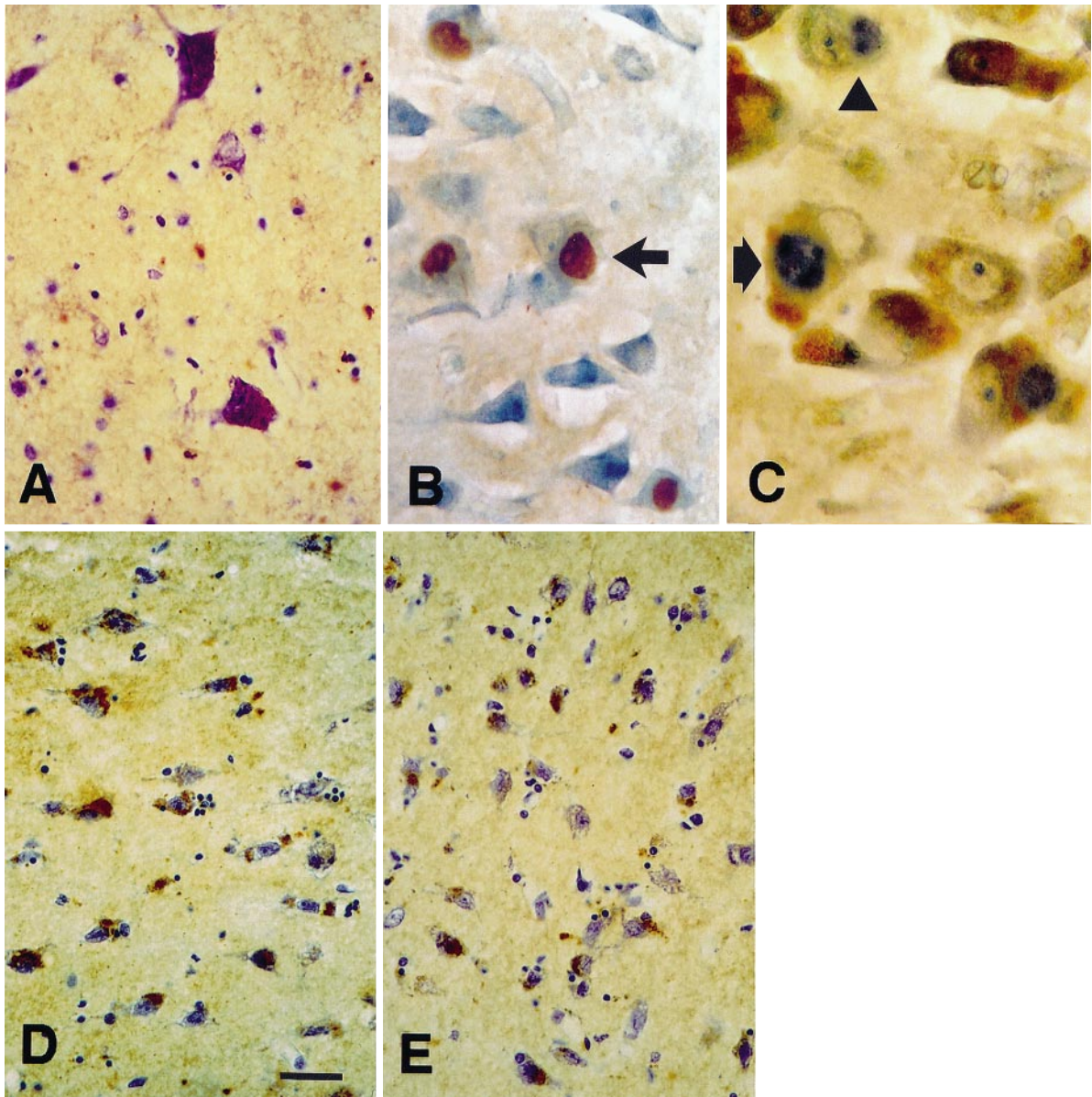


Fig. 1A Dense perinuclear PS-1 staining in a PB-free neuron in the CA1 field in a 72-year-old ND patient. **B,C** Immunocytochemical visualization of PB-containing neurons (**B**) and double labeling visualization of PS-1-immunoreactive PB-containing neurons (**C**) in the CA1 field in a 74-year-old patient with PiD. *Arrows* indicate a PB-containing neuron (**B**) and a PS-1-immunoreactive PB-containing neuron (**C**), while *arrowhead* indicates a PB-containing neuron lacking PS-1 immunolabeling (**C**). **D,E** PS-1-immunoreactive neurons in the subiculum in a 76-year-old patient with PiD (**D**) and in a 74-year-old non-demented patient (**E**). Note the presence of higher PS-1-immunoreactive neuron densities in the PiD patient. Visualization of neurons containing PS-1 was made using a polyclonal antibody raised against the N-terminal PS-1 sequence ([5]; **A, D, E**), and PB were detected with a monoclonal antibody to the microtubule-associated protein tau (**B**) in Nissl-counterstained sections [8, 13]. Double labeling with these antibodies was performed to visualize PS-1-immunoreactive PB-containing neurons (**C**) (*PS-1* presenilin-1, *PB* Pick bodies, *ND* non-demented, *PiD* Pick's disease). *Bar A, B* 300 μ m, *C* 200 μ m, *D, E* 600 μ m

and in the hippocampal formation. These demented patients form the PiD group of this study.

All of the brains were obtained at autopsy (postmortem delay: 3–20 h), fixed in a 10% formalin solution for at least 6 weeks, and cut into 1-cm-thick coronal slices. The following cortical areas were analyzed in both ND and PiD cases (Brodmann's nomenclature): 9 in the prefrontal cortex; 22 in the superior temporal cortex; 20 in the inferior temporal cortex, the entorhinal cortex, subiculum, CA1–3 fields of the hippocampus, and hilus of the dentate gyrus. Neuronal densities were obtained from Nissl-stained sections. PS-1 expression was detected using a fully characterized polyclonal antibody raised against the N-terminal PS-1 sequence (TELPAPLSYFNRKC-NH₂; [5]) as previously described [7] (Fig. 1A). This antibody recognizes both the 46-kDa holoprotein and the 27-kDa PS-1 N-terminal derivative. Additional sections were treated with an affinity-purified and fully characterized polyclonal antibody raised against sequence 311–330 of the human PS-1 long isoform [6]. The densities of PS-1-immunoreactive neurons were obtained from Nissl-stained sections. Briefly, 12-mm-thick cryostat sections were rinsed in PBS followed by treatment for 10 min with potassium permanganate (0.25%) to mask lipofuscin fluorescence [7, 8]. After incubation overnight with the primary antibody-

Table 1 Neuron densities in ND and PiD cases. Results represent neuron number/mm³ (\pm SEM) in each area. Note the substantial decrease in total neuron densities in all areas except the hilus of the dentate gyrus in PiD cases compared to ND cases. Cortical layers are indicated by Roman numerals. Statistical analysis was performed by the Mann-Whitney U test (ND non-demented, PiD Pick's disease, PS-1 presenilin-1)

Area/layer	Nissl-stained neurons		PS-1-immunoreactive neurons	
	ND	PiD	ND	PiD
CA1	28500 \pm 1000	11539 \pm 1700 ^a	13800 \pm 603	7441 \pm 1118 ^b
CA2-3	43779 \pm 2557	25490 \pm 1234 ^a	26700 \pm 1280	18865 \pm 1231 ^b
Hilus	21441 \pm 1191	17800 \pm 1311	17667 \pm 793	12697 \pm 1221
Subiculum	26211 \pm 1200	13000 \pm 1188 ^a	14448 \pm 856	9975 \pm 435
Entorhinal II	29500 \pm 1288	10012 \pm 945 ^a	13400 \pm 926	6925 \pm 860 ^a
Entorhinal V	33330 \pm 2678	19700 \pm 4332 ^a	12996 \pm 986	11851 \pm 1664
20 II-III	37998 \pm 1215	15566 \pm 1298 ^a	20221 \pm 7000	10062 \pm 448 ^a
V-VI	40988 \pm 1290	27705 \pm 1800 ^a	14977 \pm 904	11101 \pm 672
22 II-III	34100 \pm 1133	8911 \pm 988 ^a	14366 \pm 1070	5405 \pm 410 ^a
V-VI	36666 \pm 1156	13033 \pm 1288 ^a	14908 \pm 1011	7500 \pm 650 ^a
9 II-III	42266 \pm 1500	22997 \pm 1221 ^a	16887 \pm 1046	10800 \pm 724 ^b
V-VI	50344 \pm 1389	27411 \pm 1600 ^a	14686 \pm 900	10997 \pm 725 ^c

^a $P < 0.001$, ^b $P < 0.01$, ^c $P < 0.05$

ies, sections were incubated with a peroxidase-conjugated anti-rabbit secondary antibody for 1 h followed by rinsing with PBS. The sections were treated with 3, 3'-diaminobenzidine as a chromogen and counterstained with Nissl stain [7, 8]. The percentages of PS-1-immunoreactive PB-free and PB-containing neurons were obtained in adjacent sections using double labeling with the PS-1 antibody and a monoclonal antibody to the microtubule-associated protein tau (Fig. 1B, C; [8, 13]), according to the method described by Levey et al. [18]. To examine whether antigen masking led to an underestimation of densities of PS-1-immunoreactive PB-containing neurons, adjacent sections were processed for single antigen localization and double immunolabeling in each case. Subsequently, the densities of immunoreactive neurons were estimated in each section.

Neuron densities per mm³ were estimated using the optical disector, and a systematic random sampling scheme on the available materials [7]. The percentages of PS-1-immunoreactive neurons in ND cases, as well as the percentages of PS-1-immunoreactive PB-free and PB-containing neurons in PiD cases were assessed in a 1 in 10 series of sections, 500 μ m apart, within each cortical layer for each selected area. Statistical differences in the prevalence of PS-1-containing neurons between the two diagnosis groups were assessed by the Mann-Whitney U test.

In addition to the neuropathological analysis, electrophoresis and immunoblotting for PS-1 were performed in three pairs of PiD and ND cases matched for age and gender using the polyclonal antibody raised against the N-terminal PS-1 sequence [7], as previously described [1]. Briefly, samples were loaded onto 10–20% polyacrylamide gels and subjected to SDS-PAGE. After transfer on nitrocellulose (Amersham), membranes were blocked with 5% skimmed milk and incubated with the primary antibody overnight at 4 °C. Horseradish peroxidase-conjugated antibody was used as secondary antibody and reaction product was detected using the Amersham ECL Western blotting system. The data obtained were normalized to account for shrinkage on the amount of protein loaded in PiD cases.

Results

In both ND and PiD cases, PS-1 labeling was intense in the cytoplasm of neurons, as well as in the proximal segments of basal and apical dendrites (Fig. 1A). No PS-1 immunoreactivity was detected in axons. Rare glial cells were also PS-1 positive in both ND and PiD brains. No immunostaining was observed when the primary anti-PS-1 antibody was omitted. A similar distribution of cellular immunoreactivity was observed using the antibody

against sequence 311–330 of the human PS-1 long isoform (data not shown).

Estimates of Nissl-stained sections demonstrated that PiD cases displayed consistently lower neuron densities in all cortical areas studied, except the hilus of the dentate gyrus, compared to ND cases (Table 1). The number of neurons showing PS-1 immunoreactivity in the hilus of the dentate gyrus, subiculum, and layer V of the entorhinal cortex in all PiD cases was comparable to that found in ND cases (Table 1). In the ND group, the prevalence of neurons showing PS-1 immunoreactivity varied from 29% to 82% depending on the area. In PiD cases, the percentage of PB-free neurons which contained PS-1 was significantly higher than in ND cases in all cortical areas

Table 2 PS-1 prevalence in ND and PiD cases. Results represent the percentages of PS-1-immunoreactive PB-free and PB-containing neurons in each area. Note that PiD cases displayed significantly higher PS-1 prevalence in PB-free neurons only in areas that showed significant neuronal loss (see Table 1). In PiD cases, PS-1 prevalence was significantly lower in PB-containing compared to PB-free neurons. Cortical layers are indicated by Roman numerals. Statistical analysis was performed by one-way analysis of variance (PB Pick bodies)

Area/layer	ND	PiD	
	PB-free neurons	PB-free neurons	PB-containing neurons
CA1	48.4 \pm 2.5	68.3 \pm 3.0 ^b	30.5 \pm 1.5 ^a
CA2-3	61.0 \pm 3.5	77.5 \pm 4.1 ^c	44.0 \pm 2.2 ^a
Hilus	82.4 \pm 4.1	81.8 \pm 3.6	40.7 \pm 1.6 ^a
Subiculum	55.1 \pm 3.6	76.0 \pm 7.0 ^b	21.3 \pm 1.6 ^a
Entorhinal II	51.7 \pm 2.9	70.2 \pm 5.1 ^c	24.3 \pm 1.4 ^a
Entorhinal V	39.0 \pm 2.2	62.2 \pm 7.7 ^a	16.2 \pm 2.1 ^a
20 II-III	53.1 \pm 3.0	66.6 \pm 5.7 ^c	21.7 \pm 2.6 ^a
V-VI	36.5 \pm 2.4	45.7 \pm 2.6 ^c	30.8 \pm 2.8 ^c
22 II-III	42.1 \pm 3.9	66.5 \pm 3.1 ^a	27.1 \pm 3.2 ^a
V-VI	40.7 \pm 2.5	62.7 \pm 4.7 ^a	19.6 \pm 2.0 ^a
9 II-III	40.0 \pm 1.9	50.9 \pm 3.8 ^c	16.7 \pm 1.8 ^a
V-VI	29.2 \pm 1.8	43.1 \pm 3.2 ^c	17.6 \pm 1.2 ^b

^a $P < 0.001$, ^b $P < 0.01$, ^c $P < 0.05$ compared to the percentage of PB-free neurons showing PS-1 immunoreactivity in ND cases

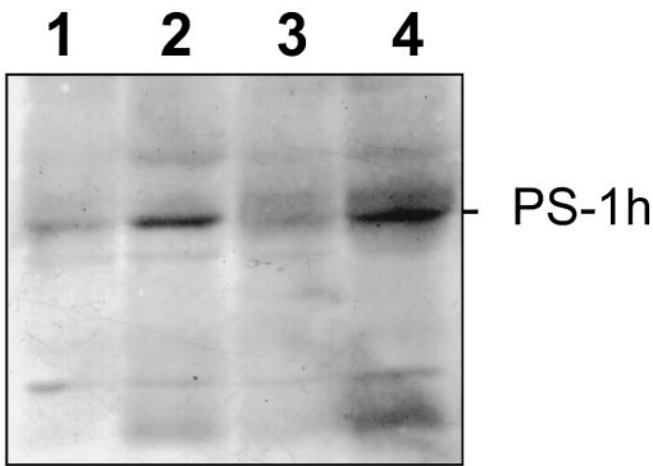


Fig. 2 Representative example of Western blot analysis for PS-1 holoprotein in areas 9 (columns 1, 2) and 20 (columns 3, 4) in two pairs of ND and PiD cases. Note that the PS-1 holoprotein levels are significantly increased in both areas in PiD cases (columns 2, 4) compared to the ND cases (columns 1, 3)

displaying neuronal loss, and varied from 43% to 82% (Table 2; Fig. 1 D, E). In the hilus of the dentate gyrus where no neuronal loss was present in PiD cases, the percentage of PS-1-immunolabeled neurons did not differ between ND and PiD cases. Only a few PB-containing neurons displayed PS-1 labeling in these areas (Fig. 1 C) so that the percentages of PS-1-immunoreactive, PB-containing neurons were 16–44% in hippocampal subdivisions and 17–31% in neocortical areas. These percentages were significantly lower than those of the PB-free neurons in all of the cortical areas in PiD brains (Table 2). The densities of PS-1-immunoreactive PB-containing neurons did not significantly differ between adjacent sections processed for single and double antigen localization.

The immunoblot analysis showed that the PS-1 holoprotein was almost absent in all of the areas studied in ND cases. There was a marked variability in the total amount of PS-1 holoprotein among PiD cases. However, a significant increase in PS-1 holoprotein levels in areas 9, 20 and 22, and hippocampus was observed in all PiD cases compared to ND cases (Fig. 2). The level of 27-kDa PS-1 N-terminal derivative was comparable between the two diagnosis groups in all of the pairs (data not shown).

Discussion

The present findings indicate that PS-1-immunoreactive neurons are relatively preserved in PiD, suggesting that the lack of PS-1 expression may be related to both neuronal loss and PB formation in the course of this disorder. The regional distribution of PS-1 in PiD cases is comparable to that reported in previous immunocytochemical studies of ND and AD cases, in that this protein is mainly located in the soma and dendrites of pyramidal neurons [2, 6, 14, 17, 19]. The PiD cases had severe cognitive deficits compatible with an advanced stage of the disease,

and a widespread loss of PS-1-labeled and -unlabeled cortical neurons. However, there were relatively higher densities of PS-1-immunoreactive, PB-free neurons in all cortical areas displaying neuronal loss in PiD compared to ND cases. This may partly due to an adaptive PS-1 overexpression which may occur in preserved neuronal subpopulations in PiD. However, the fact that the increase in PS-1-immunoreactive neurons did not occur in the hilus of the dentate gyrus, the only area which did not demonstrate substantial neuronal loss, suggests that a relative depletion in neurons lacking PS-1 takes place in PiD. This is consistent with our observations in late-onset sporadic AD cases [7], and indicates that the increase in PS-1 immunoreactivity may be the consequence of the depletion of neurons which do not express PS-1 in either dementing conditions. Interestingly, Bcl-X_L, another protein which protects against apoptotic cell death does not display a similar increase in AD cases [8], suggesting that the relative preservation of PS-1-immunoreactive neurons in neurodegenerative diseases is a specific phenomenon. However, it should be kept in mind that the densities of PS-1-immunoreactive neurons are decreased, albeit to a lesser degree than the total neuron densities, suggesting that PS-1 expression is not always sufficient to prevent neuronal death. With respect to PB, our data agree with several previous observations in AD cases and suggest that a secondary down-regulation of PS-1 follows the formation of intraneuronal neurofibrillary tangles and PB [8, 12, 23].

The neuropathological observations are also confirmed by our immunoblot analysis which show an increase in PS-1 holoprotein but not in 27-kDa PS-1 N-terminal levels in the areas displaying substantial neuronal loss in PiD cases. The PS-1 holoprotein is usually cleaved into a N-terminal derivative of 27 kDa and a C-terminal derivative of 17–18 kDa, and several studies have indicated that PS-1 mutations in AD may inhibit this process, leading to the accumulation of a non-functional form of the protein [21, 24]. However, the physiological role of this cleavage is unknown, and other lines of evidence suggest that the proteolysis of PS-1 is not a prerequisite for its function [4, 20]. The accumulation of PS-1 holoprotein in preserved neuronal populations without subsequent decrease in the levels of the N-terminal derivative in PiD is consistent with this latter hypothesis. However, such conclusions should be drawn with caution from the present study because of the limited number of cases which were analyzed by Western blot.

In this context, it is worth noting that PS-1 knockout mice show substantial neuronal loss in the cerebral cortex [22], and PS-1 mutations sensitize neurons to apoptotic death induced by trophic factor withdrawal, mitochondrial toxin and A β peptide [9, 15]. In addition, an alternative cleavage of PS-1 by a caspase-3 family protein occurs during apoptosis and may contribute to the neuronal loss in AD [16]. Our results parallel these observations in that they show an increased PS-1 immunoreactivity in surviving neurons in both late-onset AD and PiD, suggesting that this protein is activated to protect neurons at risk in a disease-independent manner. Additional molecular ge-

netic and biochemical analyses are necessary to clarify the mechanisms surrounding the cellular effects of presenilins in neurodegenerative disorders.

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