Animal Model

Age-Related Amyloid β Deposition in Transgenic Mice Overexpressing Both Alzheimer Mutant Presenilin 1 and Amyloid β Precursor Protein Swedish Mutant Is Not Associated with Global Neuronal Loss

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To analyze the relationship between the deposition of amyloid β peptides ($A\beta$) and neuronal loss in transgenic models of Alzheimer's disease (AD), we examined the frontal neocortex (Fc) and CA1 portion of hippocampus (CA1) in PSAPP mice doubly expressing AD-associated mutant presenilin 1 (PS1) and Swedish-type mutant β amyloid precursor protein (APPsw) by morphometry of $A\beta$ burden and neuronal counts. Deposition of $A\beta$ was detected as early as 3 months of age in the Fc and CA1 of PSAPP mice and progressed to cover 28.3% of the superior frontal cortex and 18.4% of CA1 at 12 months: ~20- (Fc) and ~40- (CA1) fold greater deposition than in APPsw mice. There was no significant difference in neuronal counts in either CA1 or the frontal cortex between nontransgenic (non-tg), PS1 transgenic, APPsw, and PSAPP mice at 3 to 12 months of age. In the PSAPP mice, there was disorganization of the neuronal architecture by compact amyloid plaques, and the average

number of neurons was 8 to 10% fewer than the other groups (NS, $P > 0.10$) in CA1 and 2 to 20% fewer in frontal cortex (NS, $P = 0.31$). There was no loss of total synaptophysin immunoreactivity in the Fc or dentate gyrus molecular layer of the 12-month-old PSAPP mice. Thus, although co-expression of mutant PS1 with Swedish mutant β APP leads to marked cortical and limbic $A\beta$ deposition in an age-dependent manner, it does not result in the dramatic neuronal loss in hippocampus and association cortex characteristic of AD. (*Am J Pathol* 2000, 157:331–339)

Extensive deposition of amyloid β peptides ($A\beta$) as senile plaques throughout the cerebral cortex is one of the pathological hallmarks of Alzheimer's disease (AD).¹ The observations that early-onset familial AD linked missense mutations in the β -amyloid precursor protein (β APP) and presenilin 1 and 2 (PS1 and PS2) genes increase the production^{2–6} and deposition^{7–10} of $A\beta$, especially that of the most amyloidogenic $A\beta_{42}$ species,^{11,12} strongly support a crucial role for $A\beta$ in the pathogenesis of AD. However, clinicopathological correlations in AD brains and *in vivo* studies in transgenic mouse models of cerebral amyloid deposition demonstrate that the relationship between $A\beta$ and neurotoxicity is not straightforward. The

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extensive neuronal loss in AD brains, approaching 50% in the association cortex¹³ and 70% in the CA1 portion of the hippocampus (CA1),¹⁴ is correlated with the number of neurofibrillary tangles, but not with the extent of A β deposition.^{15–18} Furthermore, multiple lines of transgenic mice overexpressing different forms of mutant β APP under various neuronal-specific promoters (ie, β APP V717F mutation of London type¹⁹ or β APP K670N/M671L mutation of Swedish type^{20,21}) do not exhibit overt neuronal loss in the cortex, and at most an average of 14% loss in CA1.²¹ It has also been reported that mice expressing mutant PS1 lose cortical and hippocampal neurons in the absence of amyloid deposition after 13 months of age.²²

Recently, double-transgenic mice expressing both mutant PS1 and mutant β APP were established.^{23,24} A β accumulation in brain was accelerated in these mice,^{23,24} supporting the notion that the primary pathogenic mechanism of mutant PS1 genes is to promote A β deposition *in vivo*. It remains unknown if neuronal loss, perhaps clinically the most important lesion in AD brains, occurs in these double-mutant transgenic mice. In this study, we examined brains of PSAPP mice²⁴ expressing both AD-associated mutant PS1 M146L and Swedish-type mutant β APP by morphometry, and analyzed the relationship between the age-related deposition of A β and neuronal loss.

Materials and Methods

Transgenic Mice

Hemizygous transgenic mice that express K670N/M671L β APP (Tg2576;²⁵ APPsw mice) and hemizygous transgenic mice expressing mutant human PS1_{M146L} (mt PS1 mice)³ were crossed, and offspring with four different genotypes, ie, mt PS1/APPsw (PSAPP mice), APPsw mice, mt PS1 mice, and nontransgenic (non-tg) mice, were obtained as previously described.^{24,26} Non-tg littermates, together with singly transgenic mt PS1 and APPsw offspring, were used as controls for the double-mutant PSAPP mice. A total of 57 (38 at the Tokyo lab and 19 at the Boston lab) mice were examined by quantitative anatomical methods. For the analysis of frontal neocortex (Fc), we used 34 mice in total: four PSAPP and four non-tg mice at ages of 3 and 12 months, two PSAPP and two non-tg mice at 6 months, one PSAPP and two non-tg at 9 months, as well as two mt PS1 and two APPsw mice at ages 3, 6, 9, and 12 months (except three mt PS1 mice at 3 months) were analyzed; in addition, a small number of very old mice (one 19 months PSAPP, two mt PS1 of 19 and 24 months, respectively, and one 24-month non-tg) also were similarly studied. An additional 19 animals were investigated at 12 months of age (five non-tg, four APPsw, six mt PS1, and four PSAPP) for amyloid burden, analysis of CA1 neurons, and synaptophysin immunohistochemistry.

Tissue Preparation

Two parallel studies were carried out in Tokyo (frontal cortex) and in Boston (CA1), using slightly different his-

tological protocols. Because the studies use complementary approaches to test the same hypotheses, we have combined the studies into a single report.

For the analysis of Fc, mice were sacrificed by cervical dislocation, and the brains were removed and fixed by immersion in 70% ethanol/150 mmol/L NaCl for 2 weeks.²⁶ The brains were then cut coronally into five blocks, dehydrated in pure ethanol, and embedded in paraffin, so that coronal sections at the levels of anterior striatum, anterior hippocampus, middle hippocampus, and brainstem appear on one slide. Serial sections were cut at 6- μ m thickness.

For the analysis of CA1, mice were anesthetized and brains removed and drop-fixed in 4% paraformaldehyde in phosphate-buffered saline, pH 7.0, for 1 day at 5°C then stored in 10% glycerol in Tris-buffered saline at 5°C before preparing 40- μ m coronal microtome sections.

Antibodies, Immunohistochemistry, and Synaptophysin Quantitation

A β immunostaining was performed using antibody 9204, an affinity-purified rabbit polyclonal antibody raised against amino acid residues 1 to 5 of human A β specifically reacting with the amino terminus of human A β starting at L-Asp,^{26–28} or biotinylated monoclonal anti-A β 3D6 1:750 (bi-3D6; gift of Elan Pharmaceuticals, South San Francisco, CA).^{19,29} Sections were immunostained by a standard avidin-biotin complex method using diaminobenzidine as chromogen, and lightly counterstained with hematoxylin.

Synaptophysin immunostaining was performed on the 40- μ m floating sections using rabbit anti-synaptophysin 1:120 (DAKO, Carpinteria, CA) and cy3-anti-rabbit IgG 1:200 (Jackson, West Grove, PA). Sections were viewed under a 20 $\times$ objective on a Nikon TE 400 microscope with a laser confocal scanning system MRC 1024 (Bio-Rad, Watford, UK). The cy3 signal (excitation wavelength 568 nm, emission wavelength 605 nm) was collected under linear conditions, accumulating six images in the photon counting mode (3% laser, iris 3.0, gain 1500, and black level 0). From each case, fields from the frontal cortex and dentate gyrus were collected from three sections. The images were transferred to NIH Image (National Institutes of Health, Bethesda, MD), where mean pixel intensity was determined for each anatomical region (frontal cortex, and inner, middle, and outer molecular layer of dentate gyrus) and adjusted for background (cy3-anti-rabbit staining alone). Synaptophysin immunoreactivity was assessed in an analysis of variance (ANOVA) by genotype and brain region. The study was powered to have an 80% probability of detecting a 25% difference in pairwise comparisons.

Morphometric Analysis

Frontal cortex neuron counts were performed in four 6- μ m cresyl violet-stained sections spaced 42 μ m apart within the anterior frontal cortex. Volume density was obtained from an anatomical region of cortex extending

Figure 1. Age-related A β deposition in PSAPP and APPsw transgenic mice. Six-micron paraffin sections of frontal neocortices from PSAPP mice at ages of 3 months (A), 6 months (B), 9 months (C), and 12 months (D), as well as those of APPsw mice at 9 months (E) and 12 months (F) were immunostained with the anti-A β antibody 9204. Original magnification: $\times 173$. G: Morphometric analysis of amyloid burden in PSAPP and APPsw transgenic mice. Percentage of amyloid burden (= percentage of total area covered by A β immunoreactivity) $\pm$ SE in the frontal neocortices of PSAPP (left column, gray) and APPsw (right column, red) mice is shown.

laterally 680 μ m from the lateral margin of the cingulate. Neuron counts were obtained from 170- μ m $\times$ 215- μ m sampling boxes, carefully ensuring that neurons were not double-counted, throughout this entire anatomical field of superior Fc. Using a 40 $\times$ objective lens, all neurons with a visible nucleolus were counted. The estimation of total Fc neurons was calculated by multiplying the volume density of the neurons by the volume of the frontal cortex with margins defined as: anterior (anterior extent of corpus callosum); posterior (posterior extent of corpus callosum); medial (medial margin of cingulate cortex); and lateral (dorsal margin of piriform/entorhinal cortex). Fc neuron counts are reported for both hemispheres.

CA1 neuron counts were performed in 40- μ m cresyl violet-stained sections spaced 240 μ m apart through the entire CA1 region of the hippocampus from one hemisphere using the optical disector technique.³⁰ The entire CA1 was systematically random sampled with approximately 25 optical disectors (25- μ m $\times$ 25- μ m sampling box with extended exclusion lines) under 100 $\times$ oil objective. CA1 neuron counts are reported for a single hemi-

sphere. The coefficient of error from the counting technique in cortex and CA1 was <0.10 ,³¹ suggesting that a minimal amount of variance in the neuronal counts is from the sampling and counting technique. Pilot experiments performed to estimate the total number of neurons in Fc using both the exhaustive sampling technique in the thin sections and the optical disector/systematic random sampling in the thick sections showed that estimates from the two tissue preparations agreed within 20% (Irizarry MC, data not shown).

Power analysis showed that studying four animals of each genotype at each time point would give a statistical power sufficient to have an 80% chance of detecting a 20% loss of neurons. Data on neuron counts of PSAPP and non-tg mice at the ages of 3 months and 12 months were statistically analyzed by StatView-J.4.11 (Abacus Concepts, Berkeley, CA).

Amyloid deposition was evaluated by quantitating the total percentage of cortical surface area covered by A β immunoreactivity (percent amyloid burden).³² In Fc, four 6- μ m sections adjacent to those used for neuron count-

Figure 2. Phosphorylated τ -positive neurites around cored plaques in 12-month-old PSAPP mice. Fc from 12-month-old PSAPP mice immunostained with a polyclonal antibody AP422 that reacts with an AD-specific phosphorylated τ epitope.

ing were immunostained with antibody 9204, and the amyloid burden was calculated and averaged using the Olympus Image analyzer SP500 (Olympus, Tokyo, Japan).^{7,8,10} For amyloid burden determination in multiple additional regions (dentate gyrus molecular layer, CA1, entorhinal cortex, cingulate cortex) of the 12-month mice, the total percentage of the regional surface area covered by amyloid deposition over one to three 40- μ m sections was determined by bi-3D6 $A\beta$ immunostaining using a Bioquant image analysis system (R&M Biometrics, Nashville, TN).^{19,29} Video images were captured and a threshold optical density was obtained that discriminated staining from background, with manual editing to eliminate artifacts.^{19,20}

Results

Age-Related $A\beta$ Deposition in the Brains of Transgenic Mice

$A\beta$ deposits were detected in the cingulate, superior frontal, and parietal neocortices and occasionally in hippocampus of 3-month-old PSAPP mice as relatively compact, round plaques of ~ 20 to $30\ \mu\text{m}$ in diameter (Figure 1A). At 6 months of age, some compact plaques of ~ 50 to $70\ \mu\text{m}$ in diameter, which were occasionally associated with multiple central cores and tiny daughter deposits, were scattered in the cortex ($23.3/\text{mm}^2$) and small, rather diffuse deposits also were observed (Figure 1B). At 9 months (Figure 1C) and 12 months (Figure 1D) of age, round, dense plaques of relatively larger sizes (~ 100 to $150\ \mu\text{m}$ in diameter) and increased number ($53.3/\text{mm}^2$ at 9 months and $60.0/\text{mm}^2$ at 12 months), and numerous small, diffuse deposits of irregular shape occupied the neuropil of the entire neocortex. The density of small deposits was increased at 12 months compared to those at 9 months. Diffuse deposits often contained neurons within the immunostained areas, although no apparent cytopathological changes were observed within the somata of these neurons by conventional histology (cresyl violet or hematoxylin and eosin; not shown) or immunohistochemistry (eg, τ immunostaining with anti-

Figure 3. Regional $A\beta$ deposition in 12-month-old PSAPP and APPsw transgenic mice. Low-power view of 40- μ m coronal sections from 12-month-old PSAPP (A) and APPsw transgenic mice (B) immunostained with the anti- $A\beta$ antibody bi-3D6. Scale bar, 1 mm. C: Percentage of amyloid burden $\pm$ SE in cingulate cortex (cing), entorhinal cortex (erc), molecular layer of dentate gyrus (dg), and CA1 of PSAPP (left column, gray) and APPsw (right column, red) mice.

body AP422 specific for phosphorylated τ at residue 422 that is specifically phosphorylated in paired helical filaments³³), although punctate AP422 immunoreactivity in the margins of cored plaques in the 12-month-old mice suggests neuritic changes harboring abnormally phosphorylated τ (Figure 2). In APPsw mice, very few isolated compact plaques were detected (~ 1 to 2 in total neocortical areas) at 6 months; some compact plaques as well as tiny deposits appeared at 9 months of age (Figure 1E), and the number of these plaques increased at 12 months (Figure 1F; $8.3/\text{mm}^2$), as previously described.^{20,25}

A detailed time-course analysis of amyloid deposition in the frontal cortex (in the region corresponding to frontal cortex neuron counts) was performed by assessing mice of ages 3 months to 12 months. By morphometry, average levels of sFc amyloid burden in PSAPP mice were 0.3% at 3 months of age, and increased with aging, reaching 28.3% at 12 months (Figure 1G). In APPsw mice, average levels of superior Fc (sFc) amyloid burden were 0.04% at 9 months and 1.4% at 12 months, respectively (Figure 1G). No $A\beta$ deposits were observed in mt PS1 mice or non-tg mice of all ages. A detailed analysis of regional amyloid burden in 40- μ m sections from multiple brain areas (molecular layer of the dentate gyrus,

Figure 4. Neuron counts in the frontal neocortices of PSAPP and control mice. Cresyl violet-stained frontal neocortices of PSAPP (A) and non-tg (B) mice at the age of 12 months. **Arrows** in A indicate amyloid plaques displacing surrounding neurons. Original magnification: $\times 105$. **C:** Total neuron number in Fc (both hemispheres) $\pm$ SE in PSAPP, APPsw, mt PS1, and non-tg mice is shown. The ANOVA was performed at 3 and 12 months.

CA1 hippocampal subfield, cingulate/retrosplenial cortex, and entorhinal cortex) was also undertaken at 12 months of age (Figure 3). Non-tg and mt PS1 transgenic mice did not contain any amyloid deposits. The APPsw mice had amyloid burdens ranging from 0.41 to 1.68% (Figure 3, B and C). PSAPP mice had a 19- to 73-fold increased amyloid deposition compared to the corresponding regions of the APPsw mice, with amyloid burdens in the range of 18 to 37% (Figure 3, A and C).

Neuron Counts in Frontal Neocortices and CA1 of Transgenic Mice

Cresyl violet-stained cerebral cortices and CA1 of PSAPP mice, especially of those in the older ages of 9 to 12 months, showed some disorganization in the cytoarchitecture of cortical neuronal layers (Figure 4A) compared to that of non-tg mice (Figure 4B), which may reflect generalized neuronal loss, focal loss of neurons within the plaques, or displacement of neurons by large, dense plaques (Figure 4A, arrows). To assess for significant neuronal loss, we performed neuron counts in the frontal cortex and CA1. No cytopathological changes, eg, nuclear condensation or neuronophagia, were observed by conventional histological examination as described above.

We used two complementary counting techniques for the analyses of Fc and CA1. These approaches take into account the degree of laminar heterogeneity present in neocortex that is not present in CA1. For Fc where the anteroposterior as well as lateral boundaries are not precisely anatomically delineated by cresyl violet staining, we chose to count the total number of neurons in the

defined region of Fc described in the Methods section, throughout the entire thickness of cortex. We performed exhaustive sampling to compensate for microheterogeneity in cell density within each cortical layer, and counted only neurons with well-defined nucleoli. Determination of total neuron counts by multiplying volume density with a defined cortical volume minimizes potential biases because of atrophy, changes in neuron size, and tissue preparation. The minimal amount of variance introduced by this sampling technique is evident by the coefficient of error for our counting technique in Fc that ranged between 0.01 to 0.08 (average: 0.025). There were no significant differences in Fc neuronal counts between non-tg, mt PS1, APPsw, and PSAPP at 3 or 12 months (Figure 4C), as assessed by two-way ANOVA (transgenic status $P = 0.312$; age $P = 0.228$). Of note, 12-month PSAPP mice had a 2 to 20% reduction in neurons compared to non-tg, PS1, or APPsw mice. In accord with these results, Fc neuron counts in substantially older mice (PSAPP 19 months: 5.26×10^6 , mt PS1 19 months: 6.51×10^6 ; 24 months: 5.85×10^6 , non-tg 24 months: 6.34×10^6 for both hemispheres) were comparable to those in animals of 3 to 12 months.

In the evaluation of neuronal counts in CA1, where the anatomical boundaries could be clearly defined and there is generally a homogeneous cell layer, we used a systematic random sampling procedure through thick sections spanning the entire CA1 region, with an average coefficient of error for the counting technique of 0.06. There were no statistically significant differences in CA1 neurons between the four groups examined at 12 months, although the PSAPP mice had an 8 to 10% reduction (ANOVA $P = 0.44$, pairwise $P = 0.11$ to 0.29) in

Figure 5. Neuron counts in the hippocampal CA1 subfield of PSAPP and control mice. Cresyl violet-stained CA1 hippocampal subfield of PSAPP (**A**) and non-tg (**B**) mice at the age of 12 months. **Arrow** in **A** indicates disorganization of surrounding neuronal lamina in the vicinity of a compact plaque; **arrowhead** in **A** indicates a glial ring surrounding a compact plaque in the stratum oriens of CA1. Scale bar, 250 μ m. **C:** Total neuron number in the entire CA1 $\pm$ SE (one hemisphere) in non-tg, APPsw, mt PS1, and PSAPP mice is shown.

neurons in CA1 compared to the non-tg, PS1 or APPsw mice (Figure 5C). In the PSAPP mice, compact amyloid plaques were associated with glial rings and occasionally disrupted the neuronal lamina (Figure 5A) relative to non-tg mice (Figure 5B).

Synaptophysin Immunoreactivity

To determine whether the cortical and hippocampal disorganization produced by amyloid plaques resulted in a net loss of synaptophysin immunoreactivity, we performed confocal quantitation of synaptophysin immunohistochemistry in the molecular layer of the dentate gyrus and the frontal cortex. There were no differences in synaptophysin immunoreactivity (Figure 6A). Cored amyloid plaques in the PSAPP mice had reduced signal in the core and were surrounded by enlarged synaptophysin immunoreactive structures consistent with dystrophic neurites (Figure 6B).

Discussion

We had two major goals: to re-examine the question of neuronal loss and amyloid deposition in transgenic models using the most robust model of amyloid deposition available, the double transgenic overexpressing mutant PS1 and mutant APP, and to evaluate the observation that overexpression of mutant PS1 alone is associated with neuronal loss. To do so, we combined two parallel studies using complementary techniques to examine frontal cortex and hippocampus. In this study, we have shown that double overexpression of mt PS1 and mt β APP, as well as the age-related massive deposition of β -amyloid caused by these transgenes, do not cause overt neuronal loss in cortices of transgenic mice with increasing age. These data are in agreement with recent morphometric studies of β APP transgenic mice showing that the entorhinal cortex, cingulate cortex, and CA1 hippocampal area of 18-month-old transgenic mice overexpressing β APP V717F (PDAPP mice), which had comparable levels of amyloid burden (21.6% in cingulate gyrus) to our

Figure 6. Synaptophysin immunoreactivity in the molecular layer of the dentate gyrus and CA1. **A:** Confocal quantitation of synaptophysin immunoreactivity by relative optical density $\pm$ SE in the outer (oml), middle (mml), and inner (iml) molecular layers of the dentate gyrus (dg) and superior frontal cortex. **B:** Synaptophysin immunostaining in frontal cortex of 12-month-old PSAPP mouse shows decreased staining in the core of plaques surrounded by synaptophysin immunoreactive dystrophic terminals (**arrows**). Scale bar, 100 μ m.

PSAPP mice (28.3%), exhibited no neuronal loss.¹⁹ In addition, the CA1 area of tg2576 overexpressing Swedish-type mutant β APP had no neuronal loss at the age of 16 months,²⁰ although another group observed neuron loss on the order of 14% in the CA1 area of APP23 mice that overexpress Swedish mutant APP.²¹ Of note, the latter mice did not show neuron loss in the neocortex despite abundant $A\beta$ deposition. Our data cannot rule out a small reduction (<20%) in neuron numbers in CA1 or cortex of the PSAPP mice, nor the possibility of very focal losses in some subset of plaques. Nonetheless, the contrast with human AD is striking: human AD cortex and CA1 contains one-third to one-fourth less amyloid (amyloid burden approximately 6%) yet have 50 to 70% neuronal loss in hippocampal and association areas.¹³ These results suggest that additional temporal and pathological factors contribute to the massive neuronal loss seen in human AD. Our study further supports the notion that $A\beta$ deposits *in vivo* do not exert massive direct neurotoxicity to neocortical neurons in transgenic mice, even when combined with the potentially accelerating effects of mutant PS1,²² although degenerative changes (eg, astrogliosis, phosphorylated τ -positive neuritic changes associated with plaques, laminar disorganization, synaptic disruption, and possible neurotoxicity of fibrillar cored plaques^{19,20}) clearly demonstrate the adverse consequences of $A\beta$ deposition in APPsw and PSAPP transgenic mice.

Our data also demonstrate that there is not a dramatic effect on global synaptophysin immunoreactivity in the APPsw and PSAPP mice. Although synaptophysin immunoreactivity and synaptic density are consistently reduced in human AD cortex and hippocampus,³⁴ the effects on synaptic density by APP transgenes, $A\beta$ production, and $A\beta$ deposition have varied in different transgenic mouse lines. Compared to non-tg mice, increased cortical synaptophysin or cholinergic terminals have been found in mice expressing human APP under the neuron-specific enolase promoter,³⁵ in young APP23 mice,³⁶ and in 8-month-old APPsw (Tg2576) mice;³⁷ reduced synaptophysin or cholinergic terminals have been identified in mice expressing hAPPV717F under the PDGF β chain promoter at both young and old ages,³⁸ and in frontal (but not parietal and entorhinal) cortex of 8-month PSAPP mice;³⁷ and no change found in synaptophysin immunoreactivity in 16-month Tg2576 mice compared to non-tg mice.²⁰ Increased synaptophysin signal from dystrophic neurites associated with compact plaques may confound reduced synaptophysin immunoreactivity from synaptic loss (Figure 6B).

In vitro experiments using primary cultured neurons have shown that the aggregated, fibrillar form of $A\beta$ is neurotoxic under culture conditions.^{39,40} Accumulating evidence also suggests that increase in the production and deposition of $A\beta$, especially that of the most aggregable $A\beta$ 42 species caused by mutations in β APP or PS genes in early-onset familial AD, is one of the most important pathogenetic factors in AD,²⁻⁷ although some nondemented aged individuals harbor abundant cortical $A\beta$ deposits without apparent neuronal damage.^{41,42} Our observation that $A\beta$ deposits including Congo Red-pos-

itive²⁴ compact plaques in PSAPP mice did not cause a generalized neuronal loss suggests that deposition of $A\beta$ alone may not be sufficient to cause further AD changes including neuron loss and neurofibrillary tangle formation.

The pathological mechanisms whereby mutations in PS genes lead to AD may not necessarily be mediated by $A\beta$ alone. Other possible mechanisms, including promotion of apoptosis, have been suggested in cells and transgenic mice overexpressing mt PS1^{43,44} or PS2.^{45,46} It has recently been reported that transgenic mice singly expressing PS1 L286V or H163R under the HeLa PDGF β 2 promoter on a FVB/N background develop hippocampal and frontal cortex neuron loss without extracellular $A\beta$ deposition at 14 to 17 months of age.²² By contrast, the transgenic line used in our studies, which overexpress PS1 M146L driven by the human PDGF β chain promoter in the C57B6 strain, did not show any neuronal loss or cytological disruption of the cortex, using an exhaustive sampling technique in Fc and a systematic random sampling technique in CA1. This suggests that the PS1 mutant effect on neuronal loss may be modified by specific mutation, promoter, and background strain, and is not *a priori* generalizable to other systems, including the PS1 M146L line used in this study which nonetheless exhibits a powerful biological effect in accelerating $A\beta$ deposition when crossed with APP transgenic mice.

Recently, a number of genetic and acquired risk factors have been implicated in neuronal death in AD brains. For example, the discovery of a familial dementing disorder, FTDP-17, linked to mutations in the τ gene strongly suggest that τ dysfunction plays a critical role in dementing disorders.^{47,48} Apoptosis⁴⁹ or brain injury⁵⁰ are highlighted as predisposing factors in AD. Crossing transgenic mice that overexpress other risk genes for AD with the PSAPP mice, or exposure of PSAPP mice to various acquired risk factors, are promising strategies to clarify the factors in addition to $A\beta$ necessary for neuronal death in AD.

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