Presenilin Mutations in Familial Alzheimer Disease and Transgenic Mouse Models Accelerate Neuronal Lysosomal Pathology

ANNE M. CATALDO, PhD, CORRINNE M. PETERHOFF, BS, STEPHEN D. SCHMIDT, BS, NICOLE B. TERIO, BS, KAREN DUFF, PhD, MARGARET BEARD, PhD, PAUL M. MATHEWS, PhD, ANDRALPH A. NIXON, MD, PhD

Abstract. The neuronal lysosomal system is a major degradative pathway, induced by cell stress and closely linked to Alzheimer disease (AD) and other neurodegenerative diseases. Here, we show that mutations of presenilin (PS) 1 and 2, which cause familial early-onset AD (FAD), induce more severe lysosomal system neuropathology in humans than does sporadic AD (SAD). Cathepsin D and B levels were higher in PS-FAD neocortex than in SAD and, unlike neurons in SAD, expressed higher levels of the cation-independent mannos-6-phosphate receptor. Lysosomal pathology was also evident in more populations of neurons in PS-FAD brains, including the less vulnerable neurons in laminae II and IV and affected neurons contained high numbers of hydrolyase-positive vesicular compartments with a broader range of abnormal morphology. In transgenic mice expressing mutant amyloid precursor protein (APPsw), introducing mutant PS1 significantly upregulated the lysosomal system in neocortical and hippocampal neurons. This upregulation, though milder in severity, resembled that seen in human PS-FAD. Accumulation of hydrolyases in dystrophic neurites in senile plaques was particularly strong, suggesting that amyloid deposition may be a stimulus for local mobilization of the lysosomal system. PS1 mice lacking the APPsw transgene also had a mild lysosomal response in some neuronal populations, which was not seen in the APPsw mice. Our findings suggest that presenilin mutations have amyloid-independent effects on the lysosomal system, which are synergistic with the lysosomal system pathology that is associated with β-amyloid.

Key Words: Animal models; Familial Alzheimer disease; Lysosomal system; Neurodegeneration; Presenilin; Proteases; Transgenic mice.

INTRODUCTION

The lysosomal system, along with the proteasome, are the 2 principal mechanisms for eliminating damaged or abnormal proteins that accumulate in many aging-related neurodegenerative diseases and may be critical to disease pathogenesis (1–6). The lysosomal system comprises several dynamically interacting vesicular compartments that are major sites for intracellular protein turnover as well as the limited proteolytic processing of certain proteins (7, 8). The major routes to the lysosome include the endocytic pathway, which is responsible for turning over or recycling membranes, and internalized proteins (9). A second pathway, autophagy, degrades intracellular organelles and cytoplasm to supply amino acids and energy for cell maintenance, repair, and remodeling following cell injury. These proteolytic processing events are carried out in acidic compartments by several dozen acid hydrolases, including various proteases, termed cathepsins, which are targeted to lysosomes after synthesis by either of 2 mannos-6-phosphate receptors, a cation-independent 215-kDa form (MPR215) and a cation-dependent 46-kDa form (MPR46) (12–14).

Dysfunction of the lysosomal system is closely tied to mechanisms of neurodegeneration. Inherited disorders of lysosome function are almost always associated with a significant neurodegenerative phenotype (15). In several pathologic settings, lysosomal proteases modify the vulnerability of neurons to degeneration and can initiate and mediate aspects of apoptosis (16, 17) or autophagic cell death (18). Various factors that are relevant to Alzheimer disease (AD) etiology, including Aβ peptide, apolipoprotein (APOE) ε4, cholesterol, and oxidative stress, decrease lysosome stability and promote lysosomal system dysfunction and cathepsin release (1, 6, 19).

At early stages of AD, neurons in vulnerable brain regions respond by greatly increasing the synthesis of lysosomal system components (3, 20–22). Higher expression of MPR46, which is among the earliest of these changes in sporadic AD (SAD), promotes delivery of lysosomal hydrolases to early endosomes and markedly increases the endosomal generation of the Aβ peptide in cell models (23, 24). As neurons become more metabolically compromised and proteasome activity declines further (25), attempts to upregulate lysosomal proteolysis accelerate. In tangle-bearing neurons, for example, the expression of most genes diminishes considerably, while that of the lysosomal hydrolase, cathepsin (Cat) D, is elevated (26, 27). The accumulation of altered hydrolase-containing compartments and associated imbalance of
proteases are conditions favorable for initiating neuronal degeneration (19).

Familial forms of AD that are caused by presenilin mutations (PS-FAD) are associated with earlier disease onset and more extensive degeneration than is SAD (28–30). Consistent with the robust neurodegeneration in PS-FAD, we show here that lysosomal system pathology is accentuated in the brains of individuals with PS-FAD compared to those with SAD. Transgenic mouse models of PS-FAD showed lysosomal system upregulation, similar to that seen in human PS-FAD, which could increase the vulnerability of neurons to degeneration. In β-amyloid depositing transgenic mice, lysosomal pathology appeared to be partly in response to neuritic plaque accumulation, and was further accentuated by mutant PS1 expression, suggesting a central role for the lysosome system in both SAD and PS-FAD.

MATERIALS AND METHODS

Human Brain Tissue

Postmortem fixed and frozen tissue obtained from 6 elderly, nondemented individuals ranging in age from 62 to 80 years was evaluated using CERAD guidelines (31), Braak staging (32), and the criteria proposed by Mirra et al (33). The tissue exhibited no gross anatomical pathology and minimal histopathological changes (isocortical and entorhinal cortices and hippocampus, 0 to 3 neuritic plaques per high-power field; 0 to 6 neurofibrillary tangles per high-power field). Another 9 age-matched cases were examined using the same criteria and diagnosed with neuropathological evidence of moderate to severe SAD (≥25 neuritic plaques per high-power field; Braak stage IV-V). Normal elderly control and SAD brain tissues were obtained from the Bronx Veteran’s Administration Medical Center and the Harvard Brain Tissue Resource Center at McLean Hospital (Belmont, MA). The magnitude of neuropathology was confirmed by histopathological inspection using Nissl stain, hematoxylin and eosin staining, Bielschowsky silver stain, and thioflavin S histo-fluorescence. Postmortem tissue from 14 confirmed cases of familial AD linked to various mutations of the presenilin gene (PS-FAD) (12 PS1-FAD cases; 2 PS2-FAD cases) were obtained from the Joseph and Kathleen Bryan Alzheimer Disease Research Center, the Duke University Medical School, the Medical College of Pennsylvania and Hahnemann University, and the National Neurological Research Species Bank/Veteran’s Administration Medical Center. All PS-FAD brains used in this study exhibited evidence of severe AD neuropathology (>30 neuritic plaques per high-power field; Braak stage IV-V).

Mice

Animals of both sexes were used in this study. Transgenic mice expressing the Swedish mutation of APP (K670M/N671L; APPswe; n = 3, 7-month-old mice; n = 3, 18- to 22-month-old mice), mutant PS1 (PS1M146L; n = 3, 7-month-old mice; n = 3, 18- to 22-month-old mice), mutant PS1 (PS1M146L; n = 3, 7-month-old mice; n = 3, 18- to 22-month-old mice) were generated as previously described (34). The presence of senile plaques was confirmed using thioflavin S histo-fluorescence, Aβ immunocytochemistry, and ultrastructural inspection.

Morphological Analyses

Immunocytochemical studies were performed on 40-μm vibratome sections of aldehyde-fixed tissue from human and mouse frontal cortex (21, 35) using a polyclonal antibody directed against human liver Cat D (Dako Corporation, Carpinteria, CA) for human studies and an affinity-purified rabbit polyclonal antibody raised against mouse brain Cat D, which was generated in this laboratory for mouse analyses. A rabbit polyclonal antibody against human liver Cat B was obtained from Cortex Biochem (San Leandro, CA) and used for human and mouse studies. MPR215 was detected using a monoclonal antibody against the luminal domain of the human protein (22) purchased from Affinity Bioreagents (Golden, CO). Acid hydrolase activity was localized in situ by enzyme histochemistry on fresh, aldehyde-fixed, 30-μm-thick sections of mouse hemisphere, which were analyzed ultrastructurally as previously described (36).

Western Blotting

Proteins extracted from homogenates obtained from frozen blocks of 6 human control, 9 SAD, and 9 PS-FAD prefrontal cortices (Brodmann A10) and nontransgenic and transgenic mouse hemi-brains (n = 3, both age groups, all genotypes) were sized by SDS-PAGE, as previously described (24), and probed for the lysosomal hydrolase, Cat D. For determination of MPR215, proteins were extracted from normal human aged controls, SAD, and PS-FAD cortical gray matter of the prefrontal cortex, and equal amounts of protein were denatured in SDS sample buffer in the absence of reducing agents. Molecular size was determined by SDS-PAGE. Following transfer to PVDF membranes, MPR215 was detected by overnight incubation using an antibody directed to the luminal domain of the protein.

RESULTS

Lysosomal System Neuropathology Is More Severe and Extensive in PS-FAD than in SAD

Antibodies to the lysosomal hydrolases Cat D and Cat B (data not shown) decorate similar populations of heterogeneously sized lysosomes and lysosome-related compartments concentrated predominantly in the perikarya and dendrites of neurons (Fig. 1). We have previously shown that these hydrolase-positive compartments in pyramidal neurons of laminae III and V of the prefrontal cortex of SAD brains exceed those seen in age-matched controls (22, 37). Notably, however, pyramidal neurons in laminae II and IV, which are known to be less vulnerable to neurodegeneration (32, 33, 38), are minimally affected in SAD (Fig. 1). By contrast, neurons in all cortical laminae of PS-FAD brains contained abnormally high numbers of cathepsin-positive compartments, including neurons within laminae II and IV. This pattern
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Fig. 1. Cat D immunoreactivity in pyramidal neurons in cortical laminae III and V in PS-FAD brains (C, arrow) is increased compared to age-matched controls (A, arrow). Similar to SAD, Cat D immunoreactivity is associated with senile plaques in the parenchyma of PS-FAD brains (B, C, double arrows and insets). In neurons of laminae II and IV, which are less vulnerable to neurodegeneration, Cat D labeling is increased in PS-FAD brains (C, F, arrowheads) compared to that in SAD (B, E, arrowheads) and control (A, D, arrowheads) cases. The increased Cat D immunoreactivity characteristically obscures the laminar appearance of the cortex in PS-FAD cases. Cat D levels determined by Western blot analysis (G) of whole brain homogenates are also increased 2-fold in PS-FAD (p < 0.001) compared to a 1.4-fold increase in SAD (p < 0.05).

was observed in all 14 PS1 and PS2 FAD brains examined. In confirmation of these findings, Western blot analyses on tissue homogenates of neocortical gray matter from the PS-FAD cases revealed a 2-fold higher level of Cat D immunoreactivity than in the corresponding brain regions from aged-matched controls (p < 0.001 for PS1 cases; p < 0.005 for PS2 cases), which was also significantly higher than the signal seen in cases of moderate to severe SAD (p < 0.01) (Fig. 1). Reactive astrocytes, which were more numerous in the PS-FAD cases and exhibited strong hydrolase immunoreactivity (not shown), were also likely to contribute to the higher Cat D protein levels in the PS-FAD brains compared to SAD and control cases.

As an index of lysosomal system activation, we also investigated the expression of MPR215, which was localized to vacuolar compartments corresponding in size and distribution to late endosomes (22) (Fig. 2). Previously, we had shown that the MPR46, but not the MPR215, is abnormally elevated in pyramidal neurons in SAD (22–24). In PS-FAD brains, however, we found immunoreactivity for the MPR215, as well as the MPR46 (not shown), increased in neurons within all neocortical layers (Fig. 2). Western blot analysis confirmed this
Immunocytochemical analyses of prefrontal cortices demonstrate that compared to control (A, D) and SAD (B, E) brains, neurons in PS-FAD brains (C, F, arrows) are more strongly immunolabeled with an antibody to MPR215. MPR215 labeling is increased in neurons in all cortical laminae in a pattern similar to that seen by Cat D immunocytochemistry (Fig. 1). Western blot analysis (G) demonstrates a nearly 2-fold elevation in MPR215-immunoreactive protein levels in PS-FAD cases compared to SAD and control cases, which do not differ.

Presenilin Mutations Promote Lysosomal System Abnormalities in β-Amyloid Depositing Transgenic Mice

Transgenic mice overexpressing human APP containing the Swedish mutation (APPswe; K670N/M671L) deposit Aβ peptide beginning at 9 to 12 months of age and achieve a significant β-amyloid plaque burden in the cingulate cortex, entorhinal cortex, and hippocampus by 18 to 20 months (39). Seven-month-old mice APPswe mice exhibited no detectable increase in Cat D and Cat B (data not shown) levels when compared to age-matched non-transgenic mice. At 18 to 22 months of age, APPswe mice displayed modestly increased Cat D and Cat B (data not shown) immunoreactivity associated with senile plaques, but no changes in immunolabeling for these proteases were seen in neuronal perikarya compared to the same neuronal groups from control mice (Fig. 3).

When a transgene encoding human PS1M146L was overexpressed in the APPswe background (PS1/APPswe mice) (34), Cat D (Fig. 4B, E) and Cat B (data not shown) immunolabeling was increased in neurons throughout the neocortical and entorhinal mantles and the hippocampus by 7 months of age. Neurons in these regions contained significantly greater numbers of...
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Fig. 3. Cat D immunoreactivity and lysosomal morphology (single arrows) in an 18-month-old APPswe transgenic mice (B, D) is similar to that seen in an age-matched, nontransgenic mice (A, C). In the APPswe mice, however, Cat D immunolabeling was associated with senile plaques (B, double arrows), which were not found in the brains of aged nontransgenic mice.

hydrolase-positive lysosomes compared to the same cell populations in age-matched APPswe and wild-type mice. These increases were more marked in 18- to 22-month-old PS1/APPswe mice (Fig. 4C, F–I), particularly throughout the cingulate, entorhinal, and hippocampal cortices, which contain high numbers of senile plaques at this age. The widespread increase in hydrolase immunoreactivity in all cortical laminae obscured the well-defined laminar cytoarchitectural pattern (Fig. 4C), as we observed in PS-FAD human brains (Fig. 1). Regions with lower PS1 transgene expression, such as the basal ganglia and cerebellum, displayed less extensive lysosomal alterations. In the older PS1/APPswe mice, lysosomes, as well as lipofuscin granules, were cathepsin-immunoreactive and many neurons exhibited enlarged profiles of hydrolase-containing compartments that were not seen in neurons from the older nontransgenic or APPswe transgenic mice (Fig. 4G–I). Neurons exhibiting the most abnormal Cat D patterns displayed neurodegenerative morphologies (Fig. 4I). Quantitative Western blot analyses confirmed this immunocytochemical evidence of a neuronal Cat D increase (Fig. 4I), showing an 80% increase (p < 0.005) in brain Cat D levels in PS1/APPswe mice when compared to nontransgenic mice of similar age.

Immuno- and enzyme-cytochemistry confirmed that acid-hydrolase reactivity was also heavily concentrated within thioflavin S-positive senile plaques in 18 month old PS1/APPswe transgenic mice (Fig. 5A–D). The presence of amyloid within the core of these lesions was confirmed in semithin, toluidine-stained sections by LM and in ultrathin sections by EM inspection (Fig. 5E). At the ultrastructural level, these lesions were composed of degenerating neurites containing abundant hydrolase-positive lysosomal compartments (Fig. 5E).

Because of the highly conserved nature of the mannose 6-phosphate receptors, we were unable to identify antibodies that detected the murine MPR215 for our analyses. Therefore, we sought additional evidence that increases in cathepsin immunolabeling in PS1/APPswe mice were reflective of a more generalized effort by the neurons to upregulate the lysosomal system by examining acid phosphatase, a nonproteolytic lysosomal hydrolase whose targeting is MPR-independent (40). In PS1/APPswe mice, ultrastructural analyses combined with enzyme cytochemistry for acid phosphatase activity revealed increased numbers of acid phosphatase-positive lysosome system-related compartments throughout the cell body (Fig. 6A). Additionally, acid hydrolase activity was consistently seen in the trans-most sacculus of the Golgi apparatus and frequently in the intermediate sacculi in the PS1/APPswe mice (Fig. 6C) but not in APPswe or nontransgenic mice. This partial distribution of acid phosphatase within the Golgi apparatus is consistent with increased hydrolase biosynthesis and lysosomal system

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Fig. 4. Cat D immunoreactivity is increased in neurons in layer III of the cingulate neocortex (arrows) of 7-month-old PS1/APPswe transgenic mice (E) compared to that in nontransgenic mice (D). Cat D immunoreactivity is increased further in 18- to 22-month-old PS1/APPswe mice (C, F; compare with Fig. 3B and E). In PS1/APPswe mice, Cat D immunocytochemistry revealed various vesicular morphologies. These include abnormally large Cat D-positive compartments in intact neurons (G, arrowheads) to neurons where Cat D labeling completely filled the cell (H, I, arrowheads). Cat D immunolabeling in PS1/APPswe mice, as in AD brain, is also detected in senile plaques in the brain parenchyma (B, C, double arrows). Western blot analysis (J) of brain homogenates (40 μg/lane) from hemi-brains of nontransgenic (NTg), APPswe (APP), and PS1 (each at n = 3) ranging in age from 18 to 22 months showed that both the 42-kDa and 34-kDa species of Cat D were increased, and nearly 2-fold higher levels of the 34-kDa Cat D-immunoreactive protein in tissue from PS1/APPswe (PA) mice of the same age (1.8 ± 0.1) compared to NTg (1.0 ± 0.1; n = 3; p < 0.005) (K).

DISCUSSION

Our studies demonstrate that the presenilin mutations in PS-FAD and transgenic mouse models of PS-FAD are associated with more severe lysosomal neuropathology than that which is seen in SAD, adding to evidence that PS-FAD is also associated with more severe...
β-amyloidosis and neurodegeneration (28–30). The introduction of a mutant PS1 transgene into the APPswe background strongly promoted a lysosomal system response in neurons of the neocortex and hippocampus, 2 regions that exhibit progressive β-amyloid deposition and neurodegenerative change. Moreover, the PS1 transgene alone induced a mild form of the lysosomal response in cortical and hippocampal regions of these mice, which lack β-amyloid pathology. By contrast, APPswe mice exhibited no discernible cathepsin-immunoreactive vesicular abnormalities in neurons and milder lysosomal responses compared to PS1/APPswe mice in neurites within senile plaques. That expression of mutant PS1 alone results in a neuronal lysosomal system response suggests that (at least in part) a mutant presenilin-driven effect on the lysosomal system can be independent of β-amyloid deposition. The more extensive lysosomal pathology seen in the PS1/APPswe mice suggests that this β-amyloid-independent effect can act in synergy with the β-amyloid-related upregulation of the neuronal lysosomal system seen in SAD and, to a more limited extent, in the β-amyloid depositing APPswe mice.

Although these β-amyloid depositing mouse models do not exhibit the cytoskeletal pathology typical of AD, they do display the characteristic neuritic plaque pathology and associated astrogliosis. While the lysosomal disturbance in the PS1/APPswe mouse remained milder than that seen in the human disease, where neurodegeneration is more extensive, many parallels in the lysosomal system alterations were seen. A loss of the laminate pattern of hydrolase staining in the neocortex was evident in both human PS-FAD and the PS1/APPswe mouse. In older PS1/APPswe mice, the neurons exhibiting the most abnormal lysosomal morphologies displayed more frequent and severe degenerative changes. Consistent with our observation of lysosomal hydrolase-rich degenerating neurons, recent studies have demonstrated a significant loss of hippocampal neurons in the CA1 of PS1/APPswe mice at 17 to 20 months of age and, to a lesser extent, in PS1 transgenic mice at the same age (44).

Although the mechanism of neuronal cell death in AD or AD mouse models has not been established, cathepsin
over-expression is implicated in both necrotic and caspase-independent apoptotic mechanisms of cell death (1, 2, 17). Cat D is an essential mediator of necrotic cell death in response to calcium injury in *C. elegans* neurons (2). In several cell types, Cat D mediates apoptosis induced by various apoptotic stimuli (45) and promotes apoptosis when overexpressed (17, 46). Cat B is essential for initiating apoptosis in other cells challenged by oxidative agents (46–48). Selectively compromising lysosomal membrane stability (leading to cathepsin enzyme leakage into the cytosol) initiates apoptotic or necrotic cell death, depending upon the conditions (45, 49). Cumulatively, these findings suggest that the lysosomal abnormalities observed here could play a key role in the neurodegenerative process in AD and may contribute to the more severe and widespread neuronal loss seen in PS-FAD, where lysosomal system pathology is particularly robust.

Our current results, together with earlier studies, highlight important differences between SAD and PS-FAD with respect to the pattern of endosomal-lysosomal system involvement. We have previously shown that disturbances of endosome function are the earliest detectable disease-specific alteration in SAD, Down syndrome, and early-onset FAD caused by APP mutations, but are absent in FAD due to presenilin mutations (50, 51). These endosomal abnormalities are promoted by the APOEe4 allele (51) and are associated with increased Aβ production through the endocytic pathway when modeled in cell lines (24, 52). In the absence of an apparent increase in the endocytic pathway’s activity in PS-FAD (51), presenilin mutations may result in extensive lysosome system pathology independently of the endocytic pathway. This raises the possibility that mutant presenilin may alter the function of autophagy, the other major pathway to the lysosome. Both presenilin and nicastrin, another component of the presenilin complex critical for γ-secretase activity, have been reported to be localized in part to lysosomes (53, 54). We have recently demonstrated that presenilin and nicastrin are most highly enriched within the autophagic vacuoles of the lysosomal system and that the autophagic pathway is a significant cellular pathway for Aβ peptide generation (55, 56). Together with these recent studies, our findings suggest an important link between lysosomal system alterations and the expression of mutant presenilin, potentially mediated through autophagy. Given the importance of the lysosomal system to a neuron’s survival and susceptibility to insult, such alterations in lysosomal system function are likely to be an important mechanism of neuronal cell vulnerability and death in AD.

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**Fig. 7.** Compared to nontransgenic mice (A, D), 7-month-old PS1 transgenic mice (B, E) show a modest elevation of Cat D immunoreactivity (arrows) in some neocortical neurons. At 18 months of age (C, F) Cat D immunoreactivity is further elevated in PS1 transgenic mice within the same neuronal populations (F, arrows). Western blot analyses (G) of brain homogenates (40 μg/lane) of the cingulate cortex dissected from five 14-month-old PS1 transgenic mice showed a 20% increase in the 34-kDa (mature) species of Cat D compared to age-matched nontransgenic (NTg) control mice (NTg mean = 1.0 ± 0.1; PS1 mean = 1.2 ± 0.1; p < 0.05).
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REFERENCES

36. Cataldo AM, Paskevich PA, Kominami E, Nixon RA. Lysosomal hydrolases of different classes are abnormally distributed in brains of patients with Alzheimer disease. Proc Natl Acad Sci USA 1991;88:10998–1002

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