Wild-type Presenilin 1 Protects against Alzheimer Disease Mutation-induced Amyloid Pathology*

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Mutations in presenilin 1 (PS1) lead to dominant inheritance of early onset familial Alzheimer disease (FAD). These mutations are known to alter the γ-secretase cleavage of the amyloid precursor protein, resulting in increased ratio of Aβ42/Aβ40 and accelerated amyloid plaque pathology in transgenic mouse models. To investigate the factors that drive the Aβ42/Aβ40 ratio and amyloid pathogenesis and to investigate the possible interactions between wild-type and FAD mutant PS1, which are co-expressed in transgenic animals, we expressed the PS1 full-length and FAD mutant PS1 cDNA in the presence of the PS1 M146V mutation (PS1M146V/+) background and crossed these alleles with the Tg2576 APP transgenic mice. Introduction of the PS1 M146V mutation on Tg2576 background resulted in earlier onset of plaque pathology. Surprisingly, removing the wild-type PS1 in the presence of the PS1 M146V mutation (PS1M146V/−) greatly exacerbated the amyloid burden, and this was attributed to a reduction of γ-secretase activity rather than an increase in Aβ42. Our findings establish a protective role of the wild-type PS1 against the FAD mutation-induced amyloid pathology through a partial loss-of-function mechanism.

The pathological hallmark defining Alzheimer disease (AD)3 is the deposition of β-amyloid plaques of which the principal components are 40–42-amino acid peptides referred to as the β-amyloid peptides (Aβ), which are derived by proteolytic cleavages of the amyloid precursor protein (APP). APP is processed by at least three proteinases known as α-, β-, and γ-secretases. Both α- and β-secretases cleave APP in the extracellular domain with α-secretase cleavage occurring inside the Aβ domain and BACE1 at the amino terminus of Aβ. These proteolytic events generate APP carboxy-terminal fragments (CTF), which serve as substrates for subsequent γ-secretase processing, producing either p3 (product of α- and γ-secretases) or Aβ42 peptides (product of BACE1 and γ-secretase) (reviewed in Ref. 1). The γ-secretase activity is executed by a high molecular weight complex of which presenilin 1 (PS1) is an essential component (reviewed in Ref. 2). As such, inhibition of PS1 leads to the accumulation of APP-CTF (3–5). The γ-secretase exhibits relaxed sequence specificity (6). In addition to APP and in an extracellular cleavage-dependent manner, PS1 has been implicated in the processing of a growing list of type 1 membrane proteins including Notch (7), N- and E-cadherins (8, 9), and the tyrosinase family of proteins (Ref. 10 and reviewed in Ref. 11).

Besides the γ-sites that generate Aβ40 and Aβ42, PS1-dependent proteolysis also occurs at other positions including the ε-site (Aβ46) and ς-site (Aβ49) (12–14). Recent evidence supports a sequential cleavage model in which ε-cleavage at the cytosol-membrane junction serves as the initial cutting site followed by ε- and γ-processing within the membrane (14). Therefore, although Aβ40 and Aβ42 are measurements of specific products of γ-secretase cleavage of APP, levels of the CTF of APP as well as other PS1 substrates, which result from insufficient PS1-dependent processing, inversely correlate with total γ-secretase activity.

Mutations in PS1 lead to autosomal dominant inheritance of familial Alzheimer disease (FAD). These mutations are known to enhance the ratio of Aβ42/Aβ40 and to foster the β-amyloid plaque pathology in transgenic mouse models (15–19). Although such a pathogenic effect by the FAD mutations can be interpreted as “gain-of-function,” the following observations do not support this simple explanation. 1) The mutations spread throughout the entire molecule. Although the majority of the mutations are missense mutations, both insertion and deletion mutations have also been identified. Thus, these mutations are indicative of a haplo-insufficiency (partial loss-of-function) or dominant-negative mechanism. Consistent with this notion, several published reports document that various PS1 FAD mutants exhibit impaired γ-secretase activities on APP, Notch, and cadherins (8, 20–22). 2) The most consistent effect by the PS1 FAD mutations is not a net gain of total Aβ but rather an increase in the ratio of Aβ42/Aβ40 (16). This can result from elevated production of Aβ42, a reduction of Aβ40, or both combined. Indeed, several publications indicate that increase in Aβ42 is at the expense of reduced Aβ40 (23, 24), suggesting that the apparent gain of Aβ42 could be secondary to partial loss of cleavage at Aβ40 or other γ-secretase sites. Furthermore, because the wild-type PS1 is present in all reported studies, whether the observed changes in Aβ42/Aβ40 ratio are due to intrinsic properties of the mutant protein or the interactions between the wild type and the mutant PS1 has not been explored.

To better understand the pathogenic mechanisms of the PS1 FAD mutations, we used PS1 M146V knock-in mice in which the M146V FAD mutation was introduced into the endogenous mouse PS1 gene in an otherwise completely physiological context (25) and expressed the PS1 M146V allele either with or without the other allele of wild-type PS1 (PS1M146V+/− or PS1M146V−/−, respectively). As expected, expression of the mutant PS1 with APP transgenic mice (APP/PS1M146V+/+) led to an earlier onset of plaque pathology (26). Surprisingly, removal of the allele...
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EXPERIMENTAL PROCEDURES

Antibodies and Peptides—The rabbit polyclonal APP antibody was raised against the carboxyl-terminal 15 amino acids of human APP. Anti-A β monoclonal antibodies 4G8 and 6E10 were purchased from Signet, and anti-N-cadherin antibody was obtained from BD Transduction Laboratories. A β-(1–40) and A β-(1–42) peptides were purchased from Bachem.

Animals—The PS1 M146V knock-in (25, 27), PS1 knock-out (28), and Tg2576 APP transgenic (26) mice have been described. The resulting APP/PS1 animals were on B6SJL hybrid background. All animal experiments were performed in accordance with the Baylor College of Medicine Institutional Animal Care and Use Committee and with national regulations and policies.

Immunohistochemistry and Amyloid Load Quantification—Antibody staining on paraffin-embedded brain sections was performed as described (19). Briefly, paraformaldehyde-perfused brains were cut into 6-μm paraffin sections. The sections were deparaffinized by heating at 55 °C for 15 min and incubated in 60% formic acid for 6 min. The slides were further deparaffinized in xylene and then rinsed with 100, 95, and 70% ethanol and water. Endogenous peroxidase activity was quenched by incubating the slides in 3% H₂O₂ in methanol for 30 min. The slides were rinsed with water and Tris-buffered saline (TBS; 50 mM Tris-HCl, pH 7.5, and 250 mM NaCl). Nonspecific epitopes were blocked for 30 min with 3% normal goat serum, 0.1% Triton X-100 in TBS. Primary antibody 6E10 was diluted 1:1000 in TBS containing 2% goat serum and incubated overnight at room temperature in a humid chamber. Sections were then washed four times for 10 min each in TBS and incubated with anti-mouse secondary antibody-peroxidase-coupled streptavidin (Vectorstain ABC kit; Vector Laboratories). Pictures were taken with a Zeiss Axioskop 2 Plus microscope equipped with the AxioCAM MRC digital camera, and the images were processed with the Axiosvision 3.1 software. Images covering the entire cortex were taken (10 sections per brain, 300 μm spacing). The number of A β plaques (>5 μm) were counted manually under an Olympus microscope (CX 31). Data were reported as the total number of plaques divided by the total cortical area for each animal. For thioflavine S staining, the paraffin-embedded slices were incubated in 0.5% thioflavine S in PBS for 10 min in the dark. Fluorescent signals were visualized after decolorizing with 95% ethanol.

Five animals/genotype/age were analyzed.

Sandwich ELISA—Sandwich ELISAs for quantifying human A β40 and A β42 were performed as described using 6E10 as the capture antibody and AB112 and AB115 as detection antibodies, which recognize A β40 and A β42 specifically (29, 30). Briefly, 4× TBS buffer (v/w) was used to homogenize brain samples, which were centrifuged at 8000 × g for 1 h. The pellet was resuspended in 5 ml guanidine twice and sonicated. 20 μl of homogenate was diluted with 10× loading buffer. After centrifugation at 8000 × g for 30 min, the samples were loaded into wells for A β-peptide detection. 100 μl of 6E10 (4 μg/ml) diluted in carbonate-bicarbonate buffer, pH 9.6, was added to coated microtiter plates and incubated at 4 °C overnight. After washing with PBS containing 0.05% Tween 20 (PBST), the plates were blocked for 1 h with 200 μl of PBST with 1% bovine serum albumin. 100 μl of standards was then applied and incubated for 2 h at room temperature or 4 °C overnight. Plates were incubated with biotinylated AB112 or AB115 diluted 1:1000 and 1:500, respectively, in PBST at room temperature for 1.5 h. Neutravidin-horseradish peroxidase (Pierce) diluted 1:5000 in PBST was added and incubated for 1 h at room temperature. After washing the plate three times, 100 μl/ml 3,3’5’,5’-tetramethylenediamine solution was added and incubated for 15–30 min at room temperature. The color development was stopped by adding 100 μl/well stop solution (1 M phosphoric acid). The optical density was measured at 490 nm by a microELISA reader. The concentrations of A β40 and A β42 in the samples were calculated from the standard curve within the linear range for each plate, respectively. Six forebrain samples/genotype/age were analyzed each in triplicates.

Western Blotting—For regular Western blotting, protein extracts were separated by SDS-PAGE and electroblotted onto 0.2-μm nitrocellulose membranes (Schleicher & Schuell). Nonspecific sites were blocked by incubation in 5% (w/v) nonfat dry milk, 0.1% Tween 20. The membranes were incubated with primary antibodies followed by horseradish peroxidase-conjugated secondary antibodies and visualized with the enhanced chemiluminescence (ECL) system (Amersham Biosciences).

For A β detection, brain lysates were loaded and separated on a 16.5% mini Bicine-urea-PAGE (31). Proteins were transferred to a polyvinylidene difluoride membrane (Bio-Rad) using a semi-dry electrophoretic transfer cell. Membranes were boiled in phosphate-buffered saline for 3 min and immunoblotted with mouse monoclonal antibody 6E10 (Signet). Synthetic A β-(1–40) and A β-(1–42) peptides were used as internal standards for the identification of the corresponding A β species in the gel.

In Vitro γ-Secretase Activity—γ-Secretase activity was assayed using a peptide substrate as described (32–34). Mouse brain membrane proteins were solubilized with 1% CHAPS and incubated with APP C99. The γ-secretase-mediated 40- and 42-site cleavages were detected by ECL using the ruthenylated G2–10 and G2–11 antibodies, respectively. The nonspecific cleavage is defined as signal in the presence of 1 μM γ-secretase inhibitor L-685,458. Specific γ-secretase activity was obtained by subtracting nonspecific from total ECL signals.

RESULTS

Protective Role of Wild-type PS1 against Amyloid Pathology—To evaluate possible genetic interactions of wild-type and FAD mutant PS1 alleles on A β production and amyloid pathology, we crossed the PS1 M146V homozygous (PS1M146V/PS1M146V) mice with Tg2576 APP transgenic mice to obtain animals that were transgenic for APP and heterozygous for the PS1 M146V knock-in allele (APP/PS1M146V/+). These mice were then bred with PS1 heterozygous null animals (PS1+/−) to generate APP positive mice expressed on wild-type PS1 (+/+), PS1+/− (+/−), PS1M146V/+ (M146V/+) or PS1M146V−/− (M146V−/−) background (Fig. 1A). Females were used in all studies to eliminate potential variability contributed by gender differences.

We carried out A β immunohistochemistry (Fig. 1B) and thioflavine S staining (Fig. 1C) and quantified the amyloid plaque pathology (Fig. 1D) in the above animals at 3 months of age. As expected, no plaque deposition could be detected in APP animals expressed on either PS1 M146V/+ or PS1M146V−/− background (data not shown). Limited plaques could be seen in the cortex and hippocampus of APP/PS1M146V/+ (M146V/+), or PS1M146V−/− (M146V−/−) backgroud (Fig. 1A). Females were used in all studies to eliminate potential variability contributed by gender differences.

At 12 months, limited plaque pathology could be detected in APP/PS1M146V−/− mouse brains (+/+; Fig. 2A). Introduction of the PS1 M146V FAD mutation enhanced the amyloid load (compare +/+ with M146V+/+; Fig. 2A). Similar to results obtained from 3-month-old ani-
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M146V/+ (Fig. 2D). Overall, these results demonstrate that wild-type PS1 antagonizes the FAD mutation-induced Aβ pathology.

Removing Wild-type PS1 Leads to Reduced Aβ and Increased Aβ42/Aβ40 Ratio—To investigate the mechanisms contributing to the accelerated plaque deposition in APP/PS1M146V/+ mice, we measured human Aβ levels in forebrain samples of the above animals by sandwich ELISA (Fig. 3). Several interesting findings emerged: (i) similar to our previous analysis of endogenous Aβ (35), reduction of 50% PS1 dosage resulted in a significant decrease in both Aβ40 and Aβ42 without altering the ratio of Aβ42/Aβ40 (compare +/+ with +/−; Fig. 3, C–E); (ii) consistent with the Aβ data obtained from PS1 A264L knock-in mice (23), expression of the PS1 M146V FAD mutation was accompanied by a simultaneous reduction of Aβ40 and increase in Aβ42 (compare +/+ with M146V/++; Fig. 3, C and D); (iii) removal of the remaining wild-type PS1 from the APP/PS1M146V/+ mice led to a further reduction of Aβ40 without significantly affecting Aβ42 (compare M146V/− with M146V/++; Fig. 3, C and D); and (iv) although the increase in the ratio of Aβ42/Aβ40 in M146V/+ animals was because of a simultaneous reduction of Aβ40 and increase in Aβ42 (compare +/+ with M146V/++; Fig. 3E), further enhancement of this ratio in M146V/− mice was solely contributed by an impaired Aβ40 production as Aβ42 remained constant (compare M146V/− with M146V/++; Fig. 3E). Therefore, exacerbated plaque pathology present in M146V/− mice (Figs. 1 and 2) is likely caused by a reduced Aβ40, hence the increased ratio of Aβ42/Aβ40 rather than an increased Aβ42.

To provide further support for the sandwich ELISA data, we performed the in vitro γ-secretase activity assay by incubating the membrane proteins from the wild-type (+/+), PS1 M146V/+, or PS1 M146V/− mouse brains with the APP substrate and measuring the amount of γ-secretase-dependent Aβ40 and Aβ42 production (Fig. 4) (32–34). Consistent with our sandwich ELISA results, Aβ40 was significantly lower in PS1 M146V/+ samples compared with wild-type controls (Fig. 4A). Levels of Aβ40 were further reduced drastically in PS1 M146V/− mice when compared with both wild-type and PS1 M146V/+ animals (Fig. 4A). In contrast to the sandwich ELISA, the in vitro γ-secretase activity assay did not detect increases of Aβ42 in PS1 M146V/− animals (compare +/+ with M146V/++; Fig. 4B). Interestingly, similar to Aβ40, levels of Aβ42 were also significantly reduced by removing the wild-type PS1 (compare M146V/− with M146V/+/ and +/++; Fig. 4B). The differences in Aβ42 measurement by the two assays could be caused by differences of the detection systems. Specifically, sandwich ELISA detects the steady-state levels of Aβ and reveals the net effect of production and clearance. In contrast, the in vitro γ-secretase assay measures the rate of Aβ production. Nevertheless, the ratios of Aβ42 to Aβ40 are in total agreement between the two assays, with M146V/− > M146V/+ > +/+ (Fig. 4C), and this ratio correlates with the degree of amyloid pathology.

Impaired Total γ-Secretase Processing in PS1 M146V/− Mice—Extracellular processing of APP by α- or β-secretases generates APP carboxy-terminal fragments, which serve as substrates for γ-secretase cleavage. Therefore, although Aβ40 and Aβ42 are measurements of specific γ-secretase products, levels of APP-CTF, which result from insufficient PS-dependent processing, inversely correlate with total γ-secretase activity. Our sandwich ELISA and in vitro γ-secretase assay both documented reduced Aβ40 as well as total Aβ (Aβ40 + Aβ42) in PS1 M146V/− animals, supporting the view that exacerbated amyloid deposition by removing the wild-type PS1 is caused by an impaired γ-secretase activity. To substantiate this notion, we next determined the activity of PS1 M146V/− mice on overall PS-dependent processing. Two PS1 substrates were evaluated: APP-CTF and N-cadherin CTF.
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Western blot analysis of brain samples taken from wild-type (+/+) PS1 M146V/+ and PS1 M146V/− animals showed that the APP and N-cadherin levels remain constant among the three genotypes (Fig. 5, A and C). Although no significant differences in APP or N-cadherin CTF levels could be detected by comparing wild-type (+/+) and PS1 M146V/+ (M146V+/+) samples, the APP and N-cadherin CTFs were both significantly higher in PS1 M146V/− brains (compare M146V/− with +/+ and M146V/++; Fig. 5, A and C). These led to the increases in the ratios of APP and N-cadherin CTF to full-length (FL) protein (Fig. 5, B and D). These data lend further support that impaired β-secretase activity in PS1 M146V/− mice is the underlying mechanism for the accelerated amyloid plaque pathology.

**DISCUSSION**

Previous genetic studies of PS1 mutations were largely carried out in transgenic mice overexpressing FAD mutant human PS1 in the presence of endogenous mouse PS1. The resulting Aβ levels are thus a combined effect of wild-type and mutant PS1. The net effect of mutant PS1 alone has not been investigated. In addition, ectopic overexpression of PS1 transgenes may alter the physiological assembly of the β-secretase complex, leading to a mutation-independent effect. In the current study, we used the PS1 M146V knock-in mice in which the M146V FAD mutation was introduced into the endogenous mouse PS1 gene in an otherwise completely physiological background. We systematically investigated the effects of wild-type and mutant PS1 on Aβ production, γ-secretase processing, and amyloid plaque pathology by breeding the PS1 M146V knock-in and PS1 knock-out alleles onto a Tg2576 APP transgenic background.

Aβ immunostaining and thioflavine S fluorescence established that expression of the PS1 M146V mutation on a Tg2576 APP transgenic background (APP/PS1 M146V+/+) led to the accelerated onset of plaque deposition from 10–12 months to 3–4 months. This result is in agreement with earlier publications and sets up the base line for us to investigate the interactions between the wild-type and FAD mutant PS1 (17–19). As genetic ablation of PS1 has been shown to block Aβ production and amyloid pathology (36, 37), we expected to see ameliorated plaques by reducing PS1 dosage. Surprisingly, we found that removing the wild-type PS1 allele on PS1 M146V background (APP/PS1 M146V−) potently promoted the amyloid pathogenesis at various ages examined including 3 months (Fig. 1), 7 months (data not shown), and 12 months (Fig. 2). These results demonstrate a protective role of PS1 activity against FAD mutation-induced plaque deposition.

Three assays were employed to determine the mechanisms underlying the pathogenic effect of the PS1 M146V mutation and the protective role of wild-type PS1: sandwich ELISA, in vitro γ-secretase activity assay, and Western blot quantification of CTFs of PS1 substrates APP
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FIGURE 3. Effects of wild-type and FAD mutant PS1 on Aβ levels at 6 weeks of age. A and B, standard curves of Aβ40 and Aβ42, respectively. The sample optical density values for Aβ40 and Aβ42 range from 0.73 to 1.13 and 0.19 to 0.34, respectively. C, forebrain Aβ40. D, Aβ42 in APP/PS1+/+ (+/+), APP/PS1+/– (+/–), APP/PS1M146V/+ (M146V/+), and APP/PS1M146V−/− (M146V−/−) animals. E, normalized ratio of Aβ42/Aβ40. *, p < 0.05; **, p < 0.01 (***, p < 0.001 (Student’s t test); n.s., non-significant (p > 0.05). Asterisks above each bar value represent comparison with +/+ controls; asterisks above the bracket represent comparison between M146V/+ and M146V−/−. n = 6/group performed in triplicates.

FIGURE 4. In vitro γ-secretase assay. CHAPS-solubilized membrane proteins prepared from the wild-type (+/+), PS1 M146V/+ (M146V/+), and PS1 M146V−/− (M146V−/−) mouse brain samples were utilized for the in vitro γ-secretase activity assay. The 40- (A) and 42-site (B) cleavages were detected by G2–10 and G2–11 antibodies, respectively. C, normalized ratio of Aβ42/Aβ40. *, p < 0.05; **, p < 0.01 (Student’s t test). Asterisks above each bar value represent comparison with +/+ controls; asterisks above the bracket represent comparison between M146V/+ and M146V−/−. n = 3/group performed in triplicates.

and N-cadherin. Although sandwich ELISA and in vitro γ-secretase assay measure the specific γ-secretase cleavage products Aβ40 and Aβ42, the CTF levels inversely correlate with the total γ-secretase activity, as these are the results of insufficient processing by the γ-secretase.

Sandwich ELISA documented a simultaneous reduction of Aβ40 and increase of Aβ42 by the PS1 M146V mutation (M146V/+ versus +/++; Fig. 3, C and D), an effect also observed in the PS1 P264L knock-in line (23). Although the in vitro γ-secretase assay failed to detect an increase...
FIGURE 5. Western blot analysis of APP and N-cadherin CTF levels. A, representative Western blots of full-length APP and CTF levels in forebrains of 3-month-old wild-type (+/+), PS1 M146V/+ (M146V/+), and PS1 M146V/− (M146V/−), B, normalized APP-CTF to FL ratio (mean ± S.D.), C, N-cadherin (N-Cad) FL and CTF levels in the same set of samples. D, normalized N-cadherin CTF to FL ratio (mean ± S.D.). *p < 0.05 (Student’s t test). Asterisk above each bar value represents comparison with +/+ controls; asterisk above the bracket represent comparison between M146V/+ and M146V/−. n = 5 genotype.

In Aβ42 (M146V/+ versus +/+; Fig. 4B), both assays revealed a significant increase in the ratio of Aβ42/Aβ40 (M146V/+ versus +/+; Figs. 3E and 4C), consistent with the vast majority of published literature (reviewed in Ref. 2). However, the combined data make it difficult to decipher the primary effect by the FAD mutation as increased Aβ42/Aβ40 ratio, and accelerated plaque deposition in APP/PS1 M146V/+ animals could result from excessive production of Aβ42, a reduction of Aβ40, or both combined.

Among the two Aβ species, McGowan et al. (38) recently reported that plaques can be formed in transgenic mice expressing Aβ42 alone but not Aβ40. This finding establishes a dominant effect of Aβ42 in amyloidogenesis. However, the potential role of Aβ40 in this process remains to be addressed. Measurement of Aβ levels by sandwich ELISA documented that removal of the remaining wild-type PS1 from M146V/+ mice (APP/PS1 M146V/−) led to further reduction of Aβ40 without affecting Aβ42 (compare M146V/− with M146V/+; Fig. 3, C and D). The in vitro γ-secretase assay showed that both Aβ40 and Aβ42 were significantly reduced (M146V/− versus M146V/+; Fig. 4, A and B). The two experiments combined reveal that reduced Aβ40 rather than increased Aβ42 is the common denominator for the higher Aβ42/Aβ40 ratio (M146V/− versus M146V/+; Figs. 3E and 4C) and exacerbated plaque load in APP/PS1 M146V/− animals. Our results are consistent with the notion that the ratio of Aβ42/Aβ40 is the key factor in amyloidogenesis, and we provide evidence that increase in this ratio can be achieved by a reduction of Aβ40 without increase in Aβ42.

The reason for the apparent contrasting effects of Aβ40 and Aβ42 in amyloid deposition remains to be established. Because Aβ42 is believed to play a pivotal role in initiating the plaque pathology (39, 40), it is possible that Aβ40 interferes with the seeding process and thereby delays the plaque formation. In this regard, it will be interesting to cross Aβ40 and Aβ42 transgenic mice and determine the amyloid phenotype in these animals (38).

Measurements of Aβ values by sandwich ELISA and in vitro γ-secretase activity assay resulted in minor discrepancies (Figs. 3 and 4). Specifically, sandwich ELISA showed similar levels of Aβ42 in PS1 M146V/+ and M146V/− samples (Fig. 3D), whereas the in vitro γ-secretase activity assay revealed dramatic reduction of Aβ42 upon removal of the wild-type PS1 allele from the PS1 M146V/+ background (M146V/+ versus M146V/−; Fig. 4B). This could be caused by differences of the detection systems. Noticeably, sandwich ELISA measures the steady-state levels of Aβ, whereas the in vitro γ-secretase assay detects the rate of Aβ production. The steady-state Aβ is the net result of production and clearance. It is reasonable to speculate that Aβ42 may have a lower clearance rate, leading to its higher representation measured by sandwich ELISA. Overall, results from both assays demonstrate substantial reduction of Aβ40 production, but not Aβ42, and significant increase in the ratio of Aβ42/Aβ40 as a result of deleting the wild-type PS1 allele.

Using APP and N-cadherin CTF as readouts, which serve as substrates for γ-secretase, we show that the PS1 M146V/− mice are less efficient in processing APP and N-cadherin CTFs compared with wild-type or PS1 M146V/+ animals (Fig. 5), and thus exhibiting a partial loss-of-function on total γ-secretase activity. This result, combined with the sandwich ELISA and in vitro γ-secretase activity data, provides strong support for the notion that a reduced γ-secretase processing is the underlying mechanism for the increased Aβ42/Aβ40 ratio and exacerbated amyloid pathology. Nevertheless, an active γ-secretase complex with an imbalance of Aβ40 and Aβ42 species is likely a prerequisite for amyloidogenesis, as merely removing one allele of PS1 does not lead to changes in Aβ42/Aβ40 ratio (compare +/+ with +/+ , Fig. 3) or promote amyloid deposition (41), and genetic ablation of PS1 blocks Aβ production and amyloid pathology (36, 37).

The leading theory of AD, the “amyloid cascade hypothesis,” posits that the generation, aggregation, and/or deposition of Aβ plays a causal role in AD pathogenesis (reviewed in Ref. 42). Because PS1 is indispensable for γ-secretase-dependent Aβ generation, inhibitors of presenilin and γ-secretase are being pursued as a candidate therapy for AD. Our studies reveal a context-dependent effect of partial PS1 inhibition on amyloid pathology. Two important findings are presented: 1) the wild-type PS1 is protective against PS1 FAD mutation-induced amyloidogenesis; and 2) this protective effect is mediated through a reduced γ-secretase activity and increased ratio of Aβ42/Aβ40. Our results raise the concern that in the case of PS1 FAD patients or perhaps other undefined pathological conditions, partial inactivation of γ-secretase may lead to exacerbated rather than ameliorated amyloid pathology.

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