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APP processing and amyloid deposition in mice haplo-insufficient for presenilin 1

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Abstract

More than 70 different mutations in presenilin 1 (PS1) have been associated with inherited early onset Alzheimer's disease (AD). How all these different mutations cause disease has not been clearly delineated. Our laboratory has previously shown that co-expression of mutant PS1 in mice transgenic for amyloid precursor protein (APPswe) dramatically accelerates the rate of amyloid deposition in the brain. In our original animals mutant PS1 was substantially over-expressed, and the stabilized pool of mouse PS1 fragments was largely replaced by the human protein. In this setting the accelerated amyloid pathology in the double transgenic mice could have been due, in part, to decreased endogenous PS1 activity. To investigate this possibility, we generated APP transgenic mice with reduced levels of endogenous PS1. We find that mice harboring only one functional PS1 allele and co-expressing Mo/HuAPPswe do not develop amyloid deposits at ages comparable to mice expressing mutant PS1. We next tested whether hypo-expression of mutant PS1 could accelerate the rate of amyloid deposition using an unusual line of transgenic mice expressing PS1dE9 at low levels, finding no significant acceleration. Our findings demonstrate that the accelerated amyloid pathology, caused by so many different mutations in PS1, is clearly not a result of haplo-insufficiency that might result from inactivating mutations. Instead, our data are consistent with a gain of property mechanism.

Keywords: Presenilin 1; Alzheimer's disease; Transgenic mice; Amyloid; Neuropathology

1. Introduction

More than 70 different mutations in presenilin 1 (PS1), and its homologue PS2, can cause early onset familial Alzheimer's disease (FAD) [30] (for review see [14]). Mutations occur within each of the eight transmembrane domains, at junctions of membrane and loop domains, and within a large intracellular loop domain [14]. How all these mutations act to cause disease is an area of extensive research and ongoing controversy. It is easier to imagine that a common feature of such a large number, and so scattered a distribution, of changes would be loss of function than it is to envision the diverse mutations all resulting in the same pathogenic gain of property. Consistent with this notion, were studies in which the egg-laying defects in *Caenorhabditis elegans* lacking PS1 (called sel-12) were rescued by FAD variants of PS1. Wild type human PS1 could completely reverse the developmental defects, whereas PS1 harboring familial AD mutations resulted in only partial rescue [1]. However, similar rescue experiments conducted in PS1 null mice point to the opposite conclusion because the transgenic expression of either wild-type or mutant PS1 resulted in complete rescue of embryonic lethality in mice homozygous for a targeted endogenous PS1 allele [9,27]. The mouse experiments argue that in mammals, mutant human PS1 retains significant normal function. Moreover, to date only missense or in-frame deletion mutations in either PS1 or PS2 have been associated with FAD; no premature termination or frameshift-mutations have been identified. Collectively, these latter studies are consistent with a gain-of-property mechanism to explain the association of mutant PS1 with FAD.

Studies in both mice and cultured cells suggest that one of the gained properties of mutant human PS1 is to alter APP processing and amyloid deposition. In transgenic mice and

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human mutant PS1 gene carriers, mutations associated with FAD affect the processing of APP by γ-secretase, causing a shift in the ratio of Aβ40:42 to favor the production Aβ42 [5,8,11,29,37]. Moreover, more than 25 PS1 mutations have been tested in cell culture; compared to wild-type presenilin all elevate the ratio of Aβ42 relative to Aβ40 [24,25].

Still, how so many different mutations cause the same shift in APP processing remains unclear. Studies of presenilin biology in both vertebrates and invertebrates suggest that PS1 and PS2 compete for inclusion into a larger protein complex that contains nicastrin, Aph-1, and PEN-2 [7,12,17,20,22,32,35,43]. This complex, now designated the γ-secretase complex, participates in the proteolytic processing of APP to generate Aβ peptides which deposit in senile plaques characteristic of AD [10,16]. Thus, the current thinking is that γ-secretase complexes that contain mutant PS1 acquire a greater preference for cleaving APP at the C-terminus of Aβ42.

However, the unique biology of PS1 makes it difficult to rule out the possibility that the net effect of expressing human PS1–FAD variants in transgenic mice or cultured cells is to decrease PS1 function. As mentioned above, PS1 and PS2 compete for inclusion in a multi-protein γ-secretase complex. Moreover, transgene-derived mutant human PS1 competes with endogenous PS1 for inclusion in this complex. When human PS1 is hyper-expressed in mice, the endogenous mouse protein is replaced by the human variant [35]. Thus, in any transgenic study with mutant PS1, a net reduction of endogenous PS1 function will occur. It could be argued that FAD variants of PS1 are less functional than wild-type, and that when mutant transgenes are expressed in mice they diminish the level, and therefore the function, of the endogenous protein.

We tested genetically whether partial loss of PS1 function could account for the accelerated amyloid deposition caused by co-expression of mutant human PS1 in APPswe transgenic mice. A targeted deletion of the endogenous PS1 gene was introduced into APPswe transgenic mice, to determine if PS1 haplo-insufficiency would generate the same early onset pathology as over-expression of FAD–PS1 variants. We find that lowering endogenous PS1 by 50% does not accelerate amyloid deposition. We also studied a line of mice that expresses a very low level mutant human PS1 (HuPS1dE9) to explore how the dose of mutant protein influences amyloid deposition. Our results are consistent with the hypothesis that FAD-associated mutations in PS1 alter, rather than decrease, its function.

2. Experimental procedures

2.1. Subjects

Transgenic mice expressing a chimeric mouse/human APP695 cDNA harboring the Swedish K670M/N671L mutations (Mo/HuAPPswe; line C3-3) and human PS1with the exon-9 deletion mutation (PS1dE9; line S-9) have been described in previous publications [2–5,19]. Both of these transgenes were expressed in a vector (MoPrP.XhoI) [2], which drives high protein expression in neurons and astrocytes of the CNS [21]. Mice in which the PS1 gene was removed by targeted deletion were previously generated by Dr. Philip Wong [39], and maintained in our laboratory in a congenic C57BL/6J background. All other mice used in this study were maintained by crosses of transgene-positive males to C57BL/6J × C3H/HeJ F1 hybrid female mice from Jackson Laboratories (Bar Harbor, ME).

New lines of transgenic mice that co-express the tetracycline-suppressible transactivator (tTA) and HuPS1dE9 were generated by co-injection of two expression plasmids. One vector placed the coding sequence of the tTA into the MoPrP.Xho vector by isolating a *Hin*dIII–*Spe*I restriction fragment that contains coding sequence for tTA from pTet-tTAK (GibcoBRL, Rockville, MD, USA). The ends of this fragment were blunted with Klenow DNA polymerase followed by ligation of *Xho*I linkers and insertion into XhoI-cut MoPrP.XhoI vector. The orientation of the tTA cDNA within the construct was verified by PCR and nucleotide sequencing. Vector DNA was amplified in *Escherichia coli*, purified by cesium chloride density gradient centrifugation. The Bluescript plasmid vector sequence was removed by digestion with NotI and separated from the ∼15 kb construct fragment by electrophoresis. Vector DNA was recovered by digestion of the agarose plug with β-agarase, followed by phenol–chloroform extraction and ethanol precipitation.

A second vector placed expression of PS1dE9 under control of a promoter activated by the tetracycline-transactivator (pTet-Splice; GibcoBRL). A cDNA encoding HuPS1dE9 was excised from a construct made previously in our laboratory (pCB6-hPS1-dE9) [34] by digestion with Asp718 and BamHI, blunted with Klenow DNA polymerase and purified by agarose gel electrophoresis. The cDNA fragment was then ligated into EcoRV digested pTet-Splice. The orientation of the HuPS1dE9 cDNA within the construct was verified by restriction mapping and nucleotide sequencing. Vector DNA was then prepared for injection as described above.

The linearized MoPrP.XhoI-tTA and pTetSplice-HuPS1dE9 vectors were mixed in equal parts by weight, resulting in a molar ratio of roughly five copies of pTetSplice-HuPS1dE9 to one copy of MoPrP.tTA. The vector mixture was then injected into the pronucleus of fertilized eggs from C57BL/6J × C3HeJ F1 matings.

2.2. Genotyping of tTA/HuPS1dE9 founders and other transgenic offspring

Offspring were genotyped for the presence of each transgene by PCR amplification of genomic DNA extracted from 1 cm of tail biopsy as described previously [18]. Transgenic lines generated from the MoPrP.XhoI vector (Mo/

HuAPPswe-line C3-3, HuPS1dE9-line S-9, and tTA-line 25) were genotyped by PCR with three primers: one antisense primer matching a sequence within the 3′ UTR of the vector and the endogenous mouse PrP gene, one sense primer specific for coding sequence in the transgene, and one sense primer specific for coding sequence in the last exon of the endogenous PrP gene. Amplification of the endogenous genomic PrP sequence by the PrP sense and antisense primers provided a positive control for DNA quality and a means to estimate transgene copy numbers. The primers used were as follows: antisense, 5′-GTG GAT ACC CCC TCC CCC AGC CTA GAC C; sense for endogenous PrP, 5′-CCT CTT TGT GAC TAT GTG GAC TGA TGT CGG; sense for Mo/HuAPPswe, 5′-CCG AGA TCT CTG AAG TGA AGA TGG ATG; sense for HuPS1, 5′-CAG GTG GTG GAG CAA GAT G; and sense for tTA, 5′-GCT TAA TGA GGT CGG AAT CGA AGG. Amplification reactions were run for 35 cycles at 94 °C for 1 min, 55–65 °C for 2 min, and 72 °C for 2 min. All samples, transgenic and wild-type, give a 750 bp product from the endogenous PrP gene. In mice transgenic for MoPrP.Mo/HuAPPswe, transgene-positive samples have an additional band at 400 bp, MoPrP.HuPS1dE9 transgene-positive samples yield a 1.3 kb band, and MoPrP.tTA positive samples yield a 1.2 kb band.

Detection of pTetSplice.HuPS1dE9 transgenes was carried out by PCR with primers within the PS1dE9 cDNA (PS1 sense: 5′-CAG GTG GTG GAG CAA GAT G, tTA: GCT TAA TGA GGT CGG AAT CGA AGG; PS1 antisense: 5′-GGC TAC GAA ACA GGC TAT GGT TG; product 1.2 kb). To control for DNA quality, the PCR reactions for pTet-Splice.HuPS1dE9 also contained PrP-sense and PrP-antisense primers that amplify a 750 bp portion of the endogenous PrP gene. Genotyping of mice with targeted endogenous PS1 alleles was also based on a three-primer competitive reaction. One primer recognized PS1 genomic sequences common to both the endogenous and targeted PS1 alleles, one primer recognized sequence specific to the wild-type alleles, and a third primer recognized sequence in the neomycin gene that marks the targeted allele (primer sequences; 5′-CTT CCA TGA GCC ATT TGC TAA GTG C, 5′-AGC CAA GAA CGG CAG CAG CA, and 5′-GGT GGA TGT GGA ATG TGT GCG AGG). Reactions were amplified for 35 cycles at 94 °C for 30″, 65 °C for 90″, and 72 °C for 90″. A 450 bp PCR product results from amplification of the endogenous PS1 allele, whereas the targeted allele generates a 250 bp PCR product.

2.3. Analysis of protein expression

Expression of HuPS1dE9 and processing of Mo/HuAPPswe were analyzed by immunoblotting. Frozen hemibrains from transgenic animals were thawed and homogenized by sonication in five volumes of PBS with 5 mM EDTA and protease inhibitors (Sigma, St. Louis, MO, USA), using a probe sonicator set to 50% output for 30 s (70 W; TEKMAR, Cincinnati, OH, USA). A fraction of this homogenate was diluted with an equal volume of detergent solution (PBS plus 2% SDS, 1% deoxycholate, 1% NP-40, 5 mM EDTA, and protease inhibitor cocktail), vortexed briefly, and centrifuged for 10 min at high speed. The pellet was discarded, and the remaining supernatant used for analysis. Fifty micrograms of brain homogenate was loaded per lane onto a 4–20% gradient Tris–glycine SDS–PAGE gel (Invitrogen, Carlsbad, CA, USA) and electrophoresed for several hours in 1× TG–SDS buffer (Amresco, Solon, OH). Proteins were transferred overnight to 0.45 μm Optitran nitrocellulose (Schleicher and Schuell, Keene, NH) in 1× TG buffer (Amresco). Blots for PS1dE9 expression were blocked in PBS containing 5% non-fat dry milk powder, and incubated for 3 h at room temperature with rabbit anti-PS1NT antibody (kind gift of Dr. Gopal Thinakaran) [36] diluted 1:2500 in blocking solution. Subsequently, the blots were washed with PBS containing 0.1% Tween-20, and then incubated with Protein A conjugated to horseradish peroxidase (HRP; Sigma) diluted 1:2500 in blocking solution. After washing several times in PBS with 0.1% Tween-20, the blot was developed with enhanced chemiluminescence reagent (NEN, Boston, MA) and exposed to film.

For study of APP processing, solubilized proteins were electrophoresed in 10–20% gradient Tricine–SDS–PAGE gels (Invitrogen) with 1× Tricine–SDS buffer (Invitrogen). Proteins were transferred overnight to 0.45 μm Optitran nitrocellulose (Schleicher and Schuell) in 1× TG buffer (Amresco). The blots were blocked in TBS containing 5% non-fat dry milk powder, and incubated for 3 h at room temperature with either mouse monoclonal antibody to human Aβ (mAb 6E10; Signet Laboratories, Dedham, MA, USA) diluted 1:2500 or rabbit polyclonal antiserum to the C-terminus of APP (CT15; kind gift of Dr. Edward Koo, via Dr. Gopal Thinakaran [31]) diluted 1:2000 in blocking solution. Blots using mAb 6E10 were further incubated with a rabbit anti-mouse IgG bridging antibody diluted 1:2000. Bound antibodies were visualized by incubation with protein A/HRP and ECL as described above.

2.4. Analysis of mRNA expression

Although the data is not shown, Northern blotting of HuPS1dE9 transgene mRNA in F1 offspring from several tTA/PS1 founders was performed in the analysis of these lines. Animals were sacrificed at 2–3 months of age, and each brain hemisphere dissected and quickly frozen on dry ice. Total RNA was extracted using Trizol Reagent (Gibco BRL, Rockville, MD, USA) according to the manufacturer's recommendations. Eight to ten micrograms of total RNA from each sample was then electrophoresed in 1% MOPS–formaldehyde agarose gels, transferred to 0.45 μm Optitran nitrocellulose (Schleicher and Schuell) in 10× SSC, UV cross-linked, and then probed with radiolabeled cDNA fragments as previously described [3].

2.5. Histology

Mice were euthanized by ether inhalation and brains removed for immersion fixation in 4% paraformaldehyde/1× phosphate buffered saline (PBS). After 48 h at 4 °C, brains were transferred to PBS, dehydrated in alcohols, treated with cedarwood oil and methylsalicylate, and embedded in paraffin for sectioning. Silver impregnation histology was performed on 10 μm paraffin-embedded sections by Hirano's modification of the Bielschowsky method [42]. Briefly, sections were deparaffinized through xylene and alcohols into tap water before being placed into fresh 20% silver nitrate solution for 20 min. After washing thoroughly with distilled water, slides were immersed in 20% silver nitrate solution titrated with fresh ammonium hydroxide. After 20 min, slides were washed with ammonia water before being individually developed with 100 μl of developer (20 ml of 37% formaldehyde, 100 ml distilled water, 50 μl concentrated nitric acid, and 0.5 g citric acid) added to 50 ml of titrated silver nitrate solution. Slides were then rinsed in tap water, fixed in 5% sodium thiosulfate, and dehydrated through alcohols and xylene.

3. Results

To test whether loss of PS1 function can accelerate the rate of Aβ deposition in mice, we crossed mice expressing Mo/HuAPPswe (line C3-3 [2]) with mice heterozygous for the targeted deletion of PS1 [39]. Previous studies of PS1 hemizygous mice demonstrated that these animals express two-fold less PS1 protein than wild-type mice [39]. Mice transgenic for APPswe and hemizygous for PS1 ($n = 7$) were examined for amyloid pathology at 12 months of age. This time point falls between the very early onset seen in Mo/HuAPPswe mice co-expressing PS1dE9 (4.5–6 months of age), and the very late onset seen in Mo/HuAPPswe single transgenic animals (>18 months; line C3-3; [3,4]). The rationale being the following: If a two-fold reduction in endogenous PS1 levels caused a proportional two-fold acceleration in Aβ deposition, then initial deposits should be visible at 9 months of age in C3-3 mice hemizygous for endogenous PS1. However, no amyloid pathology was found in any of the APPswe transgenic/PS1 hemizygous animals examined at 12 months of age (Fig. 1C and D). Thus, reducing PS1 levels by 50% in APPswe transgenic mice does not recapitulate the acceleration of amyloid pathology caused by over-expression of mutant PS1 [3,4,21].

In an unrelated project, we had been working to produce mice in which expression of mutant PS1 could be regulated by tetracycline analogs using elements of the tet-off expression system of Gossen and Bujard ([15], purchased from Gibco/BRL, Rockville, MD). As described in Section 2, human PS1dE9 cDNA was inserted into the pTet-Splice vector. This construct was co-injected with a plasmid encoding the tetracycline transactivator protein under control of the mouse prion protein promoter (MoPrP.XhoI). Together, this bigenic system is designed to drive the expression of human

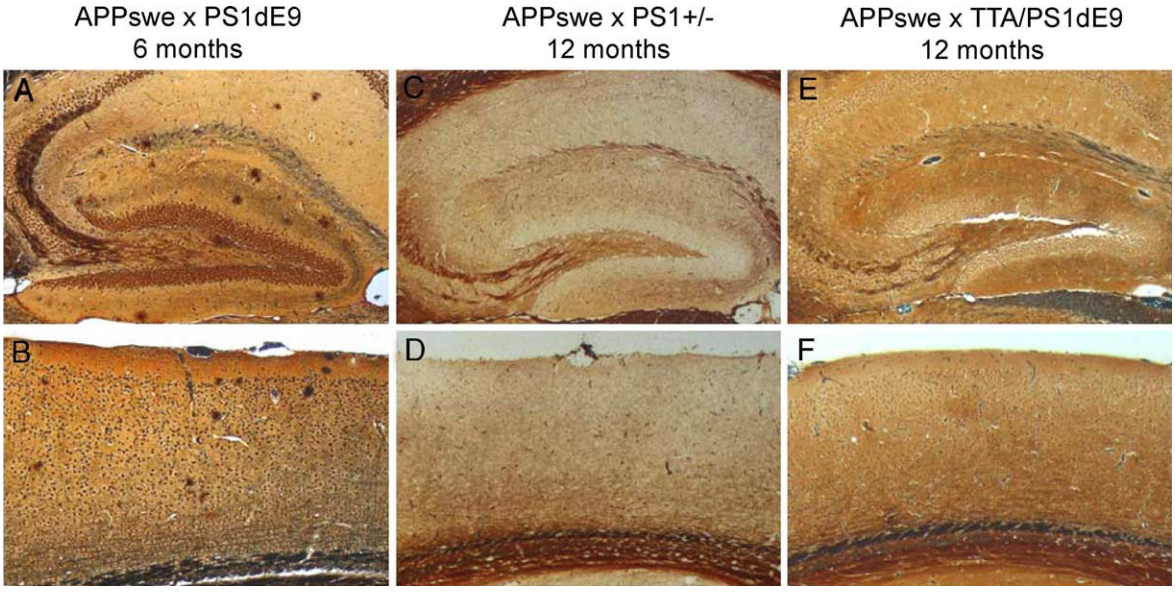

Fig. 1. Amyloid pathology in APPswe transgenic mice with altered PS1 expression. Hirano sliver stain was used to visualize amyloid aggregates in the hippocampus (A, C, and E) or cortex (B, D, and F) of transgenic mice expressing APPswe (Line C3-3). Panels A and B illustrate the acceleration of plaque pathology by co-expression of FAD-variant PS1dE9 under direct control of the mouse prion promoter (Line S-9) in mice transgenic for APPswe. As discussed in the text, panels C and D show that the loss of one PS1 allele and consequent reduction of PS1 activity does not recapitulate the effect of exogenous mutant PS1. Animals shown are 12 months of age and have no sign of amyloid pathology. Panels E and F show that accelerating amyloid pathology requires more mutant protein than produced by the low-expressing Line 25 tTA/HuPS1dE9 mice. Animals shown are 12 months of age; animals sacrificed at even later ages remained free of amyloid deposits.

PS1dE9 in a tissue distribution similar to our previously described mice harboring the MoPrP.HuPS1dE9 transgene, but in a manner that is fully suppressible by analogs of tetracycline.

This strategy for controllable HuPS1dE9 expression encountered several problems. First, despite several attempts, we have not obtained mice that stably express high levels of active tTA via the MoPrP.Xho vector. Founders were often mosaic and transmitted the transgene to offspring at a low frequency. When stable lines of mice were identified, expression of tTA was very low or quickly lost through successive generations. Second, in the few lines that did stably express tTA, the expression of mutant PS1 protein was either nonexistent or very poor. Only one line, designated Line 25, showed detectable levels of mutant PS1. However, the amount of mutant PS1 protein in Line 25 was much less than in mice where HuPS1dE9 is expressed under the direct control of the MoPrP promoter (Line S-9; Fig. 2). From immunoblot analysis of serially diluted brain homogenates (not shown), we estimate that HuPS1dE9 levels in the tTA/HuPS1dE9 Line 25 mice are less than 5% of endogenous levels. Northern analysis of whole brain homogenates likewise demonstrated that the levels of transgene mRNA in Line 25 mice was much lower than what is produced in mice where HuPS1dE9 is under the direct control of the MoPrP promoter (data not shown). We suspect that the low level of transgene-derived mRNA observed in the Line 25 mice is due to nonsense mediated decay of nascent message. Nonsense mediated decay can occur when a stop codon is found

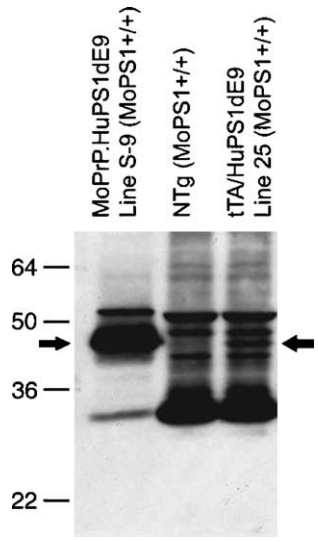

Fig. 2. Transgenic expression of HuPS1dE9 in Line 25 mice. Immunoblotting with PS1NT polyclonal antibody directed against the N-terminus of human PS1 reveals low although detectable expression of uncleaved PS1dE9 transgenic protein in brain tissue from Line 25 mice (3rd lane). Lane 1 shows the level of expression and location of the full-length protein in mice from line S-9 expressing PS1dE9 under direct control of the MoPrP promoter (1st lane, arrow to left). Only 5 μg of total brain protein from Line S-9 was loaded. In lanes 2 and 3 50 μg of protein homogenate was loaded to facilitate detection of the full-length PS1dE9 protein in the tTA/HuPS1dE9 (Line 25 mice) (3rd lane, arrow to right).

in an exon upstream of an intron [23,33]. In the pTet-Splice vector, just such a scenario is created when cDNA open reading frames are inserted into the multicloning site, which is located in exon 1, because an SV40 intron followed by a polyadenylation signal lies downstream of this exon (Gibco Life Technologies, Bethesda, MD, USA). Hence, we think it likely that transgene-derived HuPS1dE9 mRNAs are rapidly degraded by nonsense mediated decay mechanisms.

Regardless of the mechanism behind the poor expression of mutant PS1 in the tTA/PS1dE9 mice, we recognized that the mice from Line 25 might be useful to examine whether the "dose" of mutant PS1 affected its pathogenicity. Previous work had shown that mice from Mo/HuAPPswe line C3-3 co-expressing relatively high levels of HuPS1dE9 develop amyloid deposits at 4.5–6 months age [21]. Line 25 mice allowed us to test whether low levels of variant protein could accelerate amyloid deposition to the same extent. Brains from double transgenic C3-3 × Line 25 tTA/PS1dE9 mice were harvested at 6 ($n = 2$), 12 ($n = 5$), 16 ($n = 1$), and 18 ($n = 1$) months of age to look for acceleration of amyloid pathology. However, we found no sign of amyloid pathology in the tTA-PS1-dE9 (Line 25)× Mo/HuAPPswe (Line C3-3) double transgenic mice at any age examined (Fig. 1E and F). Because single transgenic Mo/HuAPPswe mice begin to develop deposits after 18 months of age [4], no later time points were observed.

The effect of low-level PS1dE9 expression in Line 25 was also examined in mice hemizygous for endogenous PS1. Again, even with less than half the normal level of wild-type PS1, mice harboring the tTA/HuPS1dE9 and Mo/HuAPPswe transgenes showed no sign of amyloid pathology at 12 months of age ($n = 5$, data not shown). Collectively, these data are consistent with the notion that the early amyloid deposition seen in mice co-expressing high levels of HuPS1dE9 with Mo/HuAPPswe is due to altered, rather than diminished, activities of the mutant PS1 protein.

Several groups have demonstrated that eliminating PS1 expression leads to dramatic accumulation (>10-fold) of both α- and β-C-terminal fragments of APP (CTFs; [6,10,26,41]). Therefore, we expected that the addition of increased substrate by Mo/HuAPPswe transgenesis in combination with PS1 haplo-insufficiency might lead to substantial increases (>5-fold) in APP-CTFs in our mice. However, such an outcome was not observed (Fig. 3B). The levels of β-CTFs (revealed by immunoblotting with mAB 6E10) in APPswe mice hemizygous for the targeted PS1 allele were not substantially different than the levels in APPswe mice with both PS1 alleles intact (Fig. 3A). As expected, the total levels of APP-CTFs (both α and β, revealed by an antibody to the C-terminus of both mouse and human APP) were substantially increased in mice expressing Mo/HuAPPswe as compared to nontransgenic controls (Fig. 3B). However, neither a- nor b-CTFs appeared to be substantially more abundant in APPswe mice hemizygous for PS1.

We also analyzed the level of APP-CTFs in the brains of mice harboring the tTA/HuPS1dE9 and APPswe trans-

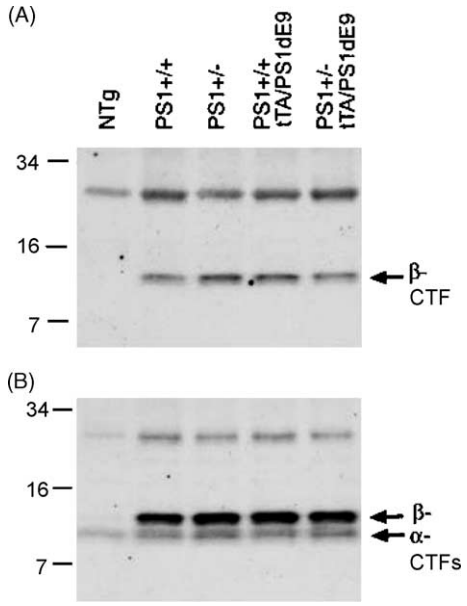

Fig. 3. APP CTF production in PS1 heterozygous mice. Immunoblots for two epitopes in the C-terminal fragments of APP reveal that CTF levels are unaffected in PS1 heterozygous mice co-expressing APPswe. (A) Antibody 6E10 recognizes Aβ residues 1–17, found only in β-CTFs. This antibody is specific for the human protein, and distinguishes endogenous from transgenic APP fragments. (B) Antibody CT15 recognizes the 15 final amino acids common to both mouse and human APP, and binds CTFs cut at β, α, or γ cleavage sites. Lane 1 shows a non-transgenic control; binding of the endogenous mouse and CTFs (arrow, CTF) is seen only with CT15. Lanes 2 and 4 show APPswe transgenic mice (Line C3-3) on a PS1 wild-type(?) background. Lanes 3 and 5 show APPswe transgenic mice heterozygous for PS1. Animals in Lanes 4 and 5 co-express low levels of tTA/HuPS1dE9 (Line 25), with no effect on APP CTF levels. Each lane contains ~5 μg of protein based on the initial weight of frozen hemibrain tissue.

genes. Again we found no dramatic differences in APP-CTFs between mice hemi or homozygous for endogenous PS1 (Fig. 3A and B). We conclude from these results that reducing PS1 levels by 50% in hemizygous animals does not dramatically affect the processing of APP-CTFs.

4. Discussion

We have studied how genetic manipulation of endogenous PS1 expression affects APP processing and amyloid deposition in APPswe transgenic mice. Our goal was to determine whether the acceleration in amyloid deposition seen in mice transgenic for FAD–PS1 variants is mimicked in mice expressing mutant APP but haplo-insufficient for endogenous PS1. We find that amyloid deposition is not markedly accelerated by PS1 haplo-insufficiency. These observations are consistent with previous studies of young APPswe/PS1hemizygous mice showing that the levels of Aβ40 and 42 peptides in the brain were no different than in APPswe mice with normal levels of endogenous PS1 [9]. Together, these data argue that the accelerated deposition of amyloid observed when mice expressing APP are crossed with mice expressing mutant PS1 is due to an altered function of the mutant protein rather than to a reduction in PS1 activity.

Studies with dominant negative variants of PS1 and chemical inhibitors of γ-secretase have revealed that APP-CTFs are probably the direct substrates for γ-secretase [6,10,13,38,40,41]. The expression of mutant APPswe in transgenic mice leads to a substantial elevation in the steady-state levels of β-CTF (see Fig. 3). We wondered whether γ-secretase activity might be near saturation in the APPswe transgenic animals, and thus if we reduced the amount of functional PS1 through targeted deletion would a further accumulation of unprocessed CTFs occur. Past work has shown that APP-CTSs accumulate more than 10-fold in PS1 null neurons [6,10,26,41]. Thus, we expected that a combination of increased substrate, through APPswe transgenesis, and diminished activity, by deletion of one PS1 allele, might lead to a substantial (>5-fold) accumulation of APP-CTFs. However, this outcome was not realized and we conclude that reducing PS1 levels by 50% does not significantly diminish the efficiency APP β-CTF processing by γ-secretase.

In previous studies of PS1 biology, we have established that PS1 undergoes endoproteolytic processing to create N- and C-terminal fragments which are the most stable species of the protein [28]. From a variety of studies, it is now clear that the more stable N- and C-terminal fragments are components of a larger protein complex that appears to contain nicastrin, Aph-1, and PEN-2, and that the complex formed by these four proteins is responsible for γ-secretase activity [7,12,17,20,22,32,43]. The interaction of PS1 with one or more proteins in this complex drives the accumulation of mature, processed, N- and C-terminal fragments of PS1, and the availability of PS1-binding partners places limits on the amount of mature PS1 that can accumulate. We have found that the accumulated steady-state level of stabilized PS1 derivatives in brains of mice expressing different levels of transgene mRNA does not increase in proportion to transgene mRNA levels [3,34]. In studies of cultured cells, we have shown that only a small fraction of full-length PS1 precursor is successfully processed; when excess full-length protein is produced, it is quickly degraded [28]. Moreover, we have previously demonstrated that mice with relatively few copies of mutant MoPrP.HuPS1 transgenes express enough full-length precursor PS1 to fully saturate the γ-secretase complex [3]. These many facets of PS1 biology had effectively prevented the study of sub-saturating levels of PS1 protein in model systems.

Our unintentional creation of a transgenic line that generated too little mutant PS1 to replace the endogenous protein provided an opportunity to study the effect of FAD variants without completely altering the composition of the γ-secretase complex. Specifically, the Line 25 MoPrP.tTA × pTet-Splice.HuPS1dE9 mice allowed us to test whether small amounts of PS1dE9 could induce the same

pathological consequence as when the γ-secretase complex is fully saturated with mutant PS1. We found that HuPS1dE9 expressed at sub-saturating levels does not induce the same magnitude of change in APP processing as occurs when the γ-secretase complex is saturated with mutant PS1. We conclude that there is no amplification of the pathogenic effect, as might occur if second messenger signaling cascades were activated. Instead, the ability of mutant PS1 to enhance amyloid pathology appears to be proportional to the amount of mutant protein generated.

We also note that we also attempted to mate the low-expressing tTA/HuPS1dE9 Line 25 into a PS1 null background for the purpose of testing whether we could rescue embryonic lethality. However, of 84 offspring from transgenic/PS1 hemizygous animals bred to non-transgenic/PS1 hemizygous mates, we failed to identify a single living weanling that was null for PS1. The numbers of offspring harboring the transgene and targeted alleles were consistent with a loss of approximately one-quarter of the transgene-positive embryos in utero. At present, we do not know whether the transgenes employed do not produce an adequate temporal or spatial pattern of expression, whether the frequency of rescue is very low [6], or whether PS1-dE9 is less able than other PS1variants to rescue the null phenotype. A simple interpretation is that hypo-saturation of the γ-secretase complex, as expected in tTA/HuPS1dE9 mice null for endogenous PS1, renders the complex incapable of efficiently processing Notch, leading to an embryonic lethal phenotype similar to PS1 deficient mice.

5. Conclusion

In summary, these experiments have focused on how mutations in PS1 may cause AD. First, we studied APPswe transgenic/PS1 hemizygous mice to test the hypothesis that the net effect of FAD-associated PS1 mutations is to reduce PS1 activity. Our findings are consistent with a model of altered function rather than lost activity. Second, we examined whether low levels of mutant PS1, below the threshold required to saturate the γ-secretase complex, would cause the same degree of accelerated amyloid pathology as occurs when γ-secretase is saturated with mutant PS1. We find that the effect of mutant PS1 is not amplified in vivo, and that the extent to which Aβ deposition is enhanced is likely proportional to the saturation of γ-secretase with mutant protein. Finally, we tested whether APP-CTFs would hyper-accumulate when the γ-secretase was challenged with elevated levels of APPswe but given only half the normal amount of endogenous PS1. We find reducing PS1 levels by 50% does not lead to dramatic accumulations of APP β-CTFs, suggesting that half of the normal level of PS1 activity is adequate to efficiently process even excess substrate. Hence, it is possible that therapies that target γ-secretase may need to reduce its activity by more than 50% in order to effectively reduce the production of Aβ.

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