A Loss of Function Mutant of the Presenilin Homologue SEL-12 Undergoes Aberrant Endoproteolysis in Caenorhabditis elegans and Increases Aβ42 Generation in Human Cells*

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The familial Alzheimer’s disease-associated presenilins (PSs) occur as a dimeric complex of proteolytically generated fragments, which functionally supports endoproteolysis of Notch and the β-amyloid precursor protein (βAPP). A homologous gene, sel-12, has been identified in Caenorhabditis elegans. We now demonstrate that wild-type (wt) SEL-12 undergoes endoproteolytic cleavage in C. elegans similar to the PSs in human tissue. In contrast, SEL-12 C60S protein expressed from the sel-12(ar131) allele is mislocalized in C. elegans, resulting in a large mutant N-terminal fragment. Neither SEL-12 wt nor C60S undergo endoproteolytic processing upon expression in human cells, suggesting that SEL-12 is cleaved by a C. elegans-specific endoproteolytic activity. The loss of function of sel-12 in C. elegans is not associated with a dominant negative activity in human cells, because SEL-12 C60S and the corresponding PS1 C92S mutation do not interfere with Notch cleavage. Moreover, both mutant variants increase the aberrant production of the highly amyloidogenic 42-amino acid version of amyloid β-peptide similar to familial Alzheimer’s disease-associated human PS mutants. Our data therefore demonstrate that the C60S mutation in SEL-12 is associated with aberrant endoproteolysis and a loss of function in C. elegans, whereas a gain of misfunction is observed upon expression in human cells.

Senile plaques composed of amyloid β-peptide (Aβ)

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‡ The abbreviations used are: Aβ, amyloid β-peptide; PS, presenilin; AD, Alzheimer’s disease; FAD, familial Alzheimer’s disease; βAPP, β-amyloid precursor protein; HEK 293, human embryonic kidney 293; CTF, C-terminal fragment; NTF, N-terminal fragment; NICD, Notch intracellular cytoplasmic domain; TM, transmembrane; STS, staurosporine; ALN, N-acetyl-leucinyl-leucinyl-norleucinyl; wt, wild-type; Bicine, N-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)glycine; PBS, phosphate-buffered saline; CHAPS, 3-(3-cholamidopropyldimethylammonio)-1-propanesulfonic acid.

invariant pathological hallmark of Alzheimer’s disease (AD). Aβ is generated by proteolytic processing from the β-amyloid precursor protein (βAPP) (1). β-Secretase, a recently identified aspartyl protease, mediates the N-terminal cleavage (2–5), whereas γ-secretase performs the putative intramembranous C-terminal cut (1). These two sequential cleavages result in the physiological generation and secretion of Aβ (1).

Familial Alzheimer’s disease (FAD) is frequently caused by mutations in the presenilin (PS) genes PS1 and PS2 (6). PSs are membrane proteins with 7 or 8 transmembrane (TM) domains (7–10). PSs undergo endoproteolysis (11), which results in the formation of a ~30-kDa N-terminal fragment (NTF) and a ~20-kDa C-terminal fragment (CTF). These fragments bind to each other and form a high molecular weight complex (12–15). Fragment formation is highly regulated allowing a limited level of expression (16, 17), which can only be slightly elevated upon transfection (11). Interestingly, ectopic overexpression of PSs results in the displacement of the endogenous PS1 and PS2 molecules (11, 16, 18).

FAD-associated PS mutations are thought to gain a pathological misfunction in the endoproteolytic processing of βAPP. Similar to the βAPP mutations, PS mutations result in the enhanced production of the highly amyloidogenic 42-amino acid variant of amyloid β-peptide (Aβ42) (6).

PSs are not only involved in the aberrant Aβ production in rare FAD cases but are also required for physiological Aβ production. A PS1 gene knock-out inhibits Aβ production and results in the accumulation of C-terminal βAPP fragments, which are thought to be the immediate precursors for the γ-secretase cleavage (19). The inhibition of γ-secretase cleavage indicates that PSs are directly involved in endoproteolysis of βAPP. This is supported by the finding that two aspartate residues located within the putative TM domains 6 and/or 7 of PS1 and PS2 are critically required for Aβ production (20–24). Moreover, Woll et al. (25) hypothesized that PSs are aspartyl proteases and therefore identical to the γ-secretase, which is strongly supported by the photoaffinity labeling of PS1 and PS2 (26, 27).

PSs do not only support the γ-secretase cleavage of βAPP but also a similar cleavage of Notch (28–30) and Irel (31). FAD-associated mutations (32) as well as mutations of residue 286 of PS1 to charged amino acids (33) and mutations of the aspartate located in TM6 and TM7 severely reduce endoproteolysis of Notch (21, 34, 35). Further evidence for a function of PSs in Notch signaling is also provided by a knock-out of the PS1 gene (28, 36, 37), which results in a developmental phenotype similar to the Notch knock-out. Moreover, genetic evidence indicates that the PS homologous gene sel-12 of the nematode
Caenorhabditis elegans is also directly involved in Notch signaling, because two mutations in sel-12 reduce the activity of a hyperactive allele of lin-12, the C. elegans Notch homologue (38). These sel-12 alleles result in an egg-laying defect (38) and a functional defect of two neurons involved in the animal’s temperature memory (39). The additional deletion of the second C. elegans PS homologue, hop-1, strongly enhances the sel-12 phenotype and results in sterility or early embryonic lethality, depending on the maternal contributions of either hop-1 or sel-12 (39–41). This is similar to the finding in mice where the additional ablation of the PS2 gene leads to a full Notch phenotype (42, 43). Both the egg-laying phenotype and the neuronal defects in C. elegans can be fully rescued by overexpression of wild-type (wt) PS1 and PS2 (44–47). However, FAD-associated PS1 or PS2 mutants exhibit only a weak activity in this genetic background, suggesting that human FAD mutations exhibit a reduced function rather than a dominant negative function (39, 44, 45). On the other hand, FAD-associated PS1 mutations fully rescue the developmental deficits of the PS1−/− mice (48, 49).

Although sel-12 function is genetically well understood (38, 39, 41, 44, 45, 50), little is known about the biochemical abnormalities, which on the molecular level interface with sel-12 activity. We therefore analyzed SEL-12 expression and investigated its endoproteolysis as well as its function in proteolytic processing of βAPP and Notch. Our data indicate that a loss of sel-12 function is associated either with a severe truncation of the resulting protein (sel-12(ar171)) or a defect in endoproteolysis (sel-12(ar131)). Moreover, the sel-12 loss of function mutation sel-12 C60S causes a gain of misfunction upon expression in human cells by increasing aberrant β42 generation.

**MATERIALS AND METHODS**

**Cell Culture and Cell Lines**—Human embryonic kidney 293 (HEK 293) cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, 200 μg/ml G418 (to select for βAPP expression), and 200 μg/ml zeocin (to select for PS1/SEL-12 expression), as well as 100 μg/ml hygromycin (to select for Notch ΔE expression). HEK 293 cells stably expressing PS1 D385A were generated as described previously (35).

**Construction of cDNAs**—The cDNAs encoding SEL-12 C60S and sel-12 C60S were constructed by polymerase chain reaction mediated mutagenesis of codon 92 of PS1 cDNA and codon 60 of sel-12 cDNA (GenBank AF171064) using appropriate primers (51). The mutant cDNAs were cloned into the expression vector pcDNA3.1/myc (Invitrogen) and sequenced to verify successful mutagenesis.

**Preparation of Protein Extracts from Cultured Cells and C. elegans**—HEK 293 cells were grown in 10 ml of PBS supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, 200 μg/ml G418 (to select for βAPP expression), and 200 μg/ml zeocin (to select for PS1/SEL-12 expression). The suspension was sonicated for 15 s at 4 °C. Upon sonication, 600 μl of PBS containing protease inhibitors (Sigma) was added and the suspension was incubated for 30 min at 4 °C. After removal of the glass beads, the extract was sonicated for 30 s at 4 °C. The suspended beads were removed by centrifugation (15,000 × g at 4 °C). Then, 2.1 ml of PBS containing protease inhibitors was added to the lysate, and SEL-12 was immunoprecipitated as described (51).

**Combined Immunoprecipitation/Western Blotting**—Cell lysates or worm extracts were immunoprecipitated using the indicated antibodies. Following gel electrophoresis, immunoprecipitated SEL-12 derivatives were identified by immunoblotting using antibody SA6848. Human PS1s were identified by immunoblotting using the monoclonal antibody PS1N (to detect the PS1 NTF), BL3D7 (to detect the PS1 CTF), or BL.HF5c (to detect the PS2 CTF). Bound antibodies were detected by enhanced chemiluminesence using standard procedures (ECL, Amersham Pharmacia Biotech).

**Induction of Apoptosis**—Apoptosis was induced by treating HEK 293 cells with 1 μM staurosporine (STS) for 6 h as described (62). To protect caspase-generated fragments from degradation, 50 μM N-acetyl-leucinyl-leucinyl-norleucinyl-alleurinal (ALLN) was added to the incubation media (55).

**Preparation of Protein Extracts from C. elegans**—A novel fragment of 24-kDa CTF obtained in the wt N2 sel-12(ar131) allele, which was not detected in the sel-12(ar171) allele (Fig. 1a). Precipitated SEL-12 derivatives were identified by immunoblotting using antibody SA6848. As a negative control we also analyzed protein extracts from HEK 293 cells transfected with human PS1 as well as worms expressing the sel-12(ar171) allele, which results in a premature stop of sel-12 translation (W225stop) and should therefore not give rise to any specific translation product detected by the antibodies used. Although, indeed, no specific sel-12 translation products were identified in C. elegans expressing the sel-12(ar171) allele (Fig. 1b), we detected an approximately 24-kDa CTF in the wt worms (N2) (Fig. 1b). Endoproteolysis of SEL-12 is consistent with the findings that PS1-derived CTFs from other species analyzed, including mice (11), zebrafish (18), and Drosophila (57, 58), are proteolytically processed as well. When we analyzed protein extracts derived from worms expressing the sel-12(ar131) allele (SEL-12 C60S), we surprisingly did not detect SEL-12 C-terminal fragments of similar molecular weight as observed in the wt worms. Instead of the ~24-kDa CTF obtained in the wt N2 worms, a novel fragment of ~32 kDa was observed (Fig. 1b). In parallel the holoprotein appeared to accumulate as well. This indicates that SEL-12 C60S is not efficiently processed in the worm and that an alternative processing activity leads to an aberrant cleavage.
Alternative Cleavage of SEL-12 C60S Occurs Close to TM7—

Based on the findings described in Fig. 1b, it was difficult to predict the site of alternative cleavage. Alternative cleavage could either occur further C-terminal producing a larger NTF or further N-terminal producing a larger CTF. In both cases the epitopes for antibodies SA6848 and 322 would be retained in the novel proteolytic product. To discriminate between these two possibilities, we performed a cyanogen bromide (CNBr) digest of isolated SEL-12 fragments. Because no methionine residues are observed within the cytoplasmic loop, we predicted that CNBr should not further digest the wt SEL-12 CTF but may result in additional cleavage products upon incubation with the larger SEL-12 C60S fragment (Fig. 2a). If the mis-cleavage would produce a larger NTF due to a C-terminal cleavage close to TM7, CNBr treatment would create a ~17-kDa fragment (corresponding to a peptide between Ala-203 and TM7). In contrast, a larger fragment would be produced by CNBr digestion (between Ala-203 and the C terminus of SEL-12) if endoproteolysis of SEL-12 C60S would occur further N-terminal (Fig. 2a).

SEL-12 loop fragments derived from the wt gene as well as from the sel-12(ar131) allele were isolated by immunoprecipitation (Fig. 2b), separated on Tris-Tricine gels and visualized by immunoblotting using antibody SA6848. Bands of interest were cut out of the gel and subsequently digested with CNBr. Similar experiments were carried out with isolated NTF and CTF of human PS1 (Fig. 2b). As shown in Fig. 2b, CNBr treatment did not reveal additional fragments, when SEL-12 fragment from the wt N2 worm was digested. This was fully confirmed by CNBr digestion of the human PS1 CTF, whereas the human PS1 NTF was sensitive to CNBr as expected (Fig. 2b). In contrast, the alternative SEL-12 proteolytic fragment gave rise to an ~17-kDa species (Fig. 2b), which was completely absent upon digestion of the SEL-12 fragment derived from the wt gene (Fig. 2b). Based on its molecular mass, the 17-kDa peptide may correspond to a CNBr fragment starting at Ala-203 within the predicted TM5 (Fig. 2a). These results demonstrate that the alternative SEL-12 fragment in mutant sel-12(ar131) worms is generated by a cleavage further C-terminal to the physiological cleavage site thus giving rise to a larger N-terminal fragment (Fig. 2c).

The Alternative Cleavage Is Not Produced by Caspases—It was previously shown that caspases can mediate alternative cleavage of PS1 and PS2 (59–62). SEL-12 indeed contains three potential caspase cleavage sites after the aspartate residues 276, 284, and 345 in the large cytoplasmic loop (Fig. 3a). Furthermore, FAD-associated mutations may induce apoptosis (63), and the SEL-12 C60S mutation behaves very similar to FAD mutations (see below). We therefore investigated whether caspases could generate the alternative fragment observed in the SEL-12 C60S worms. If SEL-12 is cleaved at Asp-345, an in vitro digest of isolated SEL-12 holoprotein could generate a fragment co-migrating with the alternative fragments observed in the sel-12(ar131) worms (Fig. 3a). Based on previous findings (55, 59–61) caspase-3 was chosen for the in vitro assay. As substrates we used the wt SEL-12 and the SEL-12 C60S holoproteins isolated from HEK 293 stably transfected with the sel-12 cDNA (see next paragraph). As shown in Fig. 3b, cleavage of wt SEL-12 as well as SEL-12 C60S by caspase-3 resulted in an alternative CTF with a lower molecular mass than the CTF derived from wt worms. Therefore, these data suggest that caspase-3 cleavage of SEL-12 occurs after either Asp-276 or Asp-284 (see Fig. 3c). To confirm this finding under in vivo conditions, we induced apoptosis in HEK 293 expressing PS1 or SEL-12 with 1 μM staurosporine (STS). To control STS-induced apoptosis we also monitored the production of the previously described (62)
alternative CTF of PS1 (Fig. 3d, lower panel). Under conditions where STS-induced apoptosis led to the generation of the caspase-generated alternative PS1 CTF, a prominent approximately 20-kDa CTF occurred in HEK 293 cells expressing SEL-12. This fragment co-migrated with a SEL-12-derived CTF, which was generated by caspase cleavage in vitro (Fig. 3d, upper panel). These results are consistent with previous results on caspase-generated CTFs of human PSs (59, 62–64).

Because the caspase-generated fragment identified by antibody SA6848 exhibits a considerably different molecular mass as the alternative fragments observed in the sel-12(ar131) worms (Fig. 3c), these data demonstrate that the alternative cleavage in the worm is not mediated by caspases induced during apoptosis (Fig. 3c; see also Fig. 2c). (Note that only the caspase cleavage of SEL-12 is observed in human cells; see next paragraph.)

Lack of Endoproteolysis of SEL-12 in Human Cells—To test if the miscleavage of SEL-12 can be observed upon expression in human cells, the wt sel-12 cDNA as well as the cDNA encoding sel-12 C60S were stably transfected into HEK 293 cells. This cell line was previously used in many studies (i.e. Refs. 21, 33, 35, 65) to investigate endoproteolysis of PSs as well as their function in Notch and APP endoproteolysis. To mimic the SEL-12 C60S mutation in human PS1, we introduced the corresponding mutation at the conserved codon 92 (PS1 C92S; Fig. 4a) and generated cell lines stably expressing this cDNA construct.

PS1 and SEL-12 derivatives were identified by a combined immunoprecipitation/Western blotting protocol. Surprisingly, this revealed no detectable endoproteolysis of SEL-12 (wt or C60S) in human cells, although high levels of the holoprotein were observed (Fig. 4b, first panel). Because worms are grown at 15–25 °C, the lack of SEL-12 endoproteolysis may be due to aberrant folding of SEL-12 at 37 °C. To test if that could be the case, HEK 293 cells were grown at 25 °C for 24 h. However, under these conditions endoproteolysis of SEL-12 was still not
observed (Fig. 4b, second panel). In contrast, human PS1 did undergo sufficient endoproteolysis under these conditions (Fig. 4b, third panel). This suggests that SEL-12 undergoes worm-specific endoproteolysis and indicates that human cells lack a component required for SEL-12 cleavage. Furthermore, introduction of the corresponding C92S mutation into human PS1 led to the generation of a C-terminal fragment co-migrating with PS1 CTFs derived from the wt cDNA (Fig. 4c). This also indicates that the failure in the endoproteolysis of mutant SEL-12 is worm-specific and can not be observed upon expression of the corresponding PS1 mutation in human cells.

We also analyzed whether ectopic expression of SEL-12 results in the replacement of endogenous PS fragments. Replacement of endogenous PS fragments is a widely observed phenomenon and thought to be an indication of a stable expression of the ectopic PS variant (11, 16). We observed that SEL-12 could at least partially replace endogenous PS1 and PS2 fragments, because derivatives of both endogenous PSs are reduced (Fig. 4c; see also Fig. 4b, lower panel). However, sel-12-mediated replacement of endogenous PSs is not as efficient as the replacement caused by ectopic PS expression (Fig. 4c).

**SEL-12 C60S and PS1 C92S Facilitate Notch1 Endoproteolysis in Human Cells**—Because SEL-12 C60S results in reduced Notch signaling in the sel-12(ar131) allele, we next analyzed whether SEL-12 C60S or the corresponding human PS1 C92S mutation interfere with Notch1 endoproteolysis in human cells. In pulse-chase experiments we then followed the production of the Notch1 intracellular cytoplasmic domain (NICD). Cells were metabolically labeled for 15 min with [35S]methionine and chased for 60 min in the presence of excess amounts of unla- beled methionine. A 60-min cold chase was chosen, because we and others previously found efficient NICD formation at this time point (21, 33, 66). Interestingly, SEL-12 C60S as well as PS1 C92S efficiently supported NICD formation like all other FAD-associated PS1 L286V were co-transfected with the Notch1 ΔE derivative described previously (21, 28, 33, 53). In pulse-chase experiments we then followed the production of the NICD. Cells were metabolically labeled for 15 min with [35S]methionine and chased for 60 min in the presence of excess amounts of unlabeled methionine. A 60-min cold chase was chosen, because we and others previously found efficient NICD formation at this time point (21, 33, 66). Interestingly, SEL-12 C60S as well as PS1 C92S efficiently supported NICD formation like all other PS derivatives (Fig. 5; left panel). From these results we conclude that, in contrast to the loss of function caused by the active site mutation (Fig. 5; right panel), the cysteine to serine mutations in TM1 do not interfere with Notch1 endoproteolysis in human cells. Moreover, neither accumulation of βAPP CTFs nor decreased secretion of Aβ was observed in cells expressing SEL-12 C60S or PS1 C92S (data not shown). Therefore, these mutations do not interfere with Notch1 and βAPP endoproteolysis in a dominant negative manner.

**A Gain of Misfunction in Human Cells**—The C60S mutation occurs at a highly conserved amino acid residue (Fig. 4a). This point mutation is therefore very similar to almost all FAD-associated point mutations, which also occur at evolutionary conserved residues and involve chemically rather subtle amino
acid exchanges (67). We therefore investigated whether the PS1 C92S mutation, which corresponds to the C60S mutation of the worm (see above) exhibits a pathological function in terms of increased Aβ42 generation. Conditioned media from metabolically labeled cells stably transfected with wt PS1, the FAD-associated PS1 L286V mutation, and the PS1 C92S mutation were collected and immunoprecipitated with antibody 3926. This antibody immunoprecipitates all Aβ species, including Aβ40 and Aβ42 (47). Immunoprecipitates were separated on a previously described gel system, which allowed the specific detection and quantitation of both Aβ species (56). Consistent with previous results (33, 68) this revealed that the FAD-associated PS1 L286V mutation increased the Aβ42/Aβ40 levels (Fig. 6a). Interestingly, the PS1 C92S mutation caused the production of very high levels of Aβ42. Quantitation revealed an increase of approximately 3-fold (Fig. 6a). The data were further confirmed by a previously described enzyme-linked immunosorbent assay (17, 18, 21, 33, 35, 47, 69) (data not shown). Therefore, the C60S homologous mutation in human PS1 behaves like a FAD-associated mutation. We next analyzed whether wt SEL-12 or the SEL-12 C60S mutation affects Aβ42 generation in human cells. Interestingly, an increased level of Aβ42 was observed upon expression of the wt cDNA. This was further elevated upon the expression of the C60S mutation (Fig. 6a). Increased Aβ42 production driven by wt SEL-12 indicated that wt SEL-12 has a pathological activity in human cells. Similar to our work on zebrafish PS1 (18), this appears to be due to several different amino acids within SEL-12 protein at positions corresponding to previously identified FAD-associated point mutations (Fig. 6b).

**DISCUSSION**

Experiments to rescue the egg-laying phenotype of C. elegans (sel-12(ar131), sel-12(ar171)) are now frequently used to test the biological activity of human PSs (44, 45, 47, 50). However, very little is known about the molecular mechanisms of the sel-12 mutant alleles. Specifically, endoproteolysis of SEL-12, the PS homologue in the worm, has so far not been investigated. Because endoproteolysis of human PS proteins appears to be an indication for functional expression and complex formation (11, 13, 14, 17, 70), we now studied the expression of wt SEL-12, SEL-12 C60S (sel-12(ar131)) and SEL-12 W225stop (sel-12(ar171)) in C. elegans.

SEL-12 undergoes endoproteolysis in C. elegans very similar to presenilin homologues of other species (18, 57, 58). As observed in other species, we found high levels of a SEL-12 CTF and only low amounts of the corresponding holoprotein. Expression of the sel-12(ar171) allele, which results in a premature translational stop at W225 in TM6, produces a truncated derivative that corresponds to a truncated NTF as suggested before (38). Because we and others have shown previously that such a fragment is unstable and biologically inactive, the W225stop mutation may correspond to a functional knock-out (17, 71, 72). In contrast the sel-12(ar131) allele is robustly expressed. However, we surprisingly found that the C60S mutation inhibited endoproteolysis at the wt cleavage site. We identified a larger NTF, which suggests that the aberrant endoproteolytic cleavage occurs much further C-terminal to the conventional cleavage site, most likely close to TM7. We had sequenced the sel-12 coding region in sel-12(ar131) worms and tested the correct mRNA length by reverse transcription-polymerase chain reaction (data not shown). Therefore, the aberrant fragment is not due to additional mutations that may result in alternative mRNA splicing. In addition, the sel-12(ar131) worms had been backcrossed extensively to remove
C60S mutation occurs at a highly conserved position very similar to the FAD-associated PS1 mutation. Indeed, the introduction of the serine to cysteine mutation at the homologous position of human PS1 resulted in a significant increase of Aβ42 generation, as it is observed in all FAD-associated PS mutations. One may therefore speculate that the PS1 C92S mutation could be found at some point in a family with early onset FAD. Strikingly, during the time this manuscript was under consideration, this mutation has been reported to occur in an Italian family (76).

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The alternative cleavage is remarkable, because the SEL-12 C60S mutation occurs far away from the site of endoproteolytic processing. This suggests that the mutation affects the structure of SEL-12, thus blocking the conventional cleavage site but making a secondary alternative site available for aberrant proteolytic processing. Interestingly, a very similar phenomenon was observed for an envelope protein of spleen necrosis virus (73). Several independent loss-of-function mutations located N-terminal from the conserved retroviral cleavage site of the protein induce aberrant endoproteolysis at a secondary site.

When sel-12 cDNAs were expressed in human cells, we did not observe any detectable endoproteolysis. It should be emphasized that heterologous overexpression of zebrafish PS1 in human cells allowed normal endoproteolysis (18). Interestingly, the SEL-12 holoprotein was active in Aβ generation, because wt SEL-12 as well as SEL-12 C60S increased the levels of Aβ42 generation very similar to the FAD-associated point mutation PS1 L286V. This is remarkable, because recently it was claimed that the uncleaved holoprotein of PSs is a proteolytically inactive zymogene (26). However, together with previous findings (69, 76) this appears to be unlikely, because at least some uncleaved PS derivatives can support aberrant Aβ42 generation and do not inhibit Notch endoproteolysis in a dominant negative manner like the active site mutations (35, 69, 23). The failure of SEL-12 to undergo endoproteolysis in human cells may be due to the lack of sequence conservation at the endoproteolytic cleavage site (Fig. 7; Refs. 69, 74, 75). This suggests that the endoproteolytic activity of human cells does not recognize SEL-12. However, because SEL-12 induces Aβ42 generation in human cells (and is therefore pathologically active), our data suggest that endoproteolysis of PSs and γ-secretase activity are not necessarily correlated.

The C60S mutation occurs at a highly conserved position very similar to the FAD-associated PS1 mutation. Indeed, the introduction of the serine to cysteine mutation at the homologous position of human PS1 resulted in a significant increase of Aβ42 generation, as it is observed in all FAD-associated PS mutations. One may therefore speculate that the PS1 C92S mutation could be found at some point in a family with early onset FAD. Strikingly, during the time this manuscript was under consideration, this mutation has been reported to occur in an Italian family (76).
