Molecular Dissection of Domains in Mutant Presenilin 2 That Mediate Overproduction of Amyloidogenic Forms of Amyloid β Peptides

INABILITY OF TRUNCATED FORMS OF PS2 WITH FAMILIAL ALZHEIMER’S DISEASE MUTATION TO INCREASE SECRETION OF Aβ42

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Taisuke Tomita‡, Shinya Tokuhira‡, Tadafumi Hashimoto‡, Keiko Aiba‡, Takaomi C. Saido§, Kei Maruyama‡, and Takeshi Iwatsubo‡

From the ‡Department of Neuropathology and Neuroscience, Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo 113-0033, Japan, the §Laboratory for Proteolytic Neuroscience, Brain Science Institute, RIKEN, Wako, Saitama 351-0198, Japan, and the ¶Laboratory of Neurochemistry, National Institute for Physiological Sciences, Okazaki, Aichi 444-8585, Japan

Mutations in presenilin (PS) 1 or PS2 genes account for the majority of early-onset familial Alzheimer’s disease, and these mutations have been shown to increase production of species of amyloid β peptide (Aβ) ending at residue 42, i.e. the most amyloidogenic form of Aβ. To gain insight into the molecular mechanisms whereby mutant PS induces overproduction of Aβ42, we constructed cDNAs encoding mutant and/or truncated forms of PS2 and examined the secretion of Aβ42 from COS or neuro2a cells transfected with these genes. Cells expressing full-length PS2 harboring both N141I and M239V mutations in the same polypeptide induced overproduction of Aβ42, although the levels of Aβ42 were comparable with those in cells engineered to express PS2 with one or the other of these PS2 mutations. In contrast, cells engineered to express partially truncated PS2 (eliminating the COOH-terminal third of PS2 while retaining the endoproteolytic NH2-terminal fragment) and harboring a N141I mutation, as well as cells expressing COOH-terminal fragments of PS2, did not produce Aβ42, and the levels of Aβ42 were comparable with those in cells that expressed full-length, wild-type PS2 or fragments thereof. These data indicate that: (i) the Aβ42-promoting effects of mutant PS2 proteins reach the maximum level with a given single amino acid substitution (i.e. N141I or M239V); and (ii) the expression of full-length mutant PS2 is required for the overproduction of Aβ42. Hence, cooperative interactions of NH2- and COOH-terminal fragments generated from full-length mutant PS2 may be important for the overproduction of Aβ42 that may underlie familial Alzheimer’s disease.

Alzheimer’s disease (AD) is a progressive, dementing, neurological disorder characterized pathologically by an extensive neuronal loss in the cerebral cortex as well as a massive deposition of amyloid β peptides (Aβ) as senile plaques and in the walls of blood vessels (1). A subset of early-onset AD is inherited as an autosomal dominant trait, and presenilin (PS) genes were identified as the major causative genes for these early-onset familial AD (FAD). PS1 gene, which is linked to the majority of early-onset FAD located on chromosome 14 (2), and PS2 gene (3), which is responsible for a subtype of FAD linked to chromosome 1, encode homologous polytopic membrane proteins that predominantly localize to endoplasmic reticulum (4–6) and span the membrane 8 times (7). More than 40 missense mutations (8), as well as an exon 10 deletion (9) in PS1 and two missense mutations of PS2 (3, 10), thus far have been identified in pedigrees of FAD.

The physiological function of PS proteins is unknown, although recent data from studies in Caenorhabditis elegans (11, 12) and PS1 gene knock-out mice (13, 14) indicate that PS1 may play some role in Notch signaling. The mechanisms whereby mutations in PS1 or PS2 genes cause AD also remain elusive, but several lines of evidence suggest that they may lead to AD by promoting β-amyloid deposition. Amino acid substitutions, as well as an exon 10 deletion, of PS1 (15–17) and PS2 (17, 18) have been shown to increase the secretion of a species of Aβ ending at residue 42 (Aβ42), i.e. the most amyloidogenic form of Aβ (19–21). Recent findings that the secretion of Aβ from primary neurons cultured from brains of mice that lack PS1 is decreased, despite the normal level of full-length β-amyloid precursor protein (βAPP) or the amyloidogenic COOH-terminal fragment thereof, argue for the notion that PS is an important co-factor for the proteolytic processing of βAPP at the COOH terminus of Aβ termed γ-cleavage (22). However, the mechanisms whereby mutant PS proteins affect γ-cleavage and lead to the increased production of Aβ42 are unknown. Some investigators have shown the direct association of PS and βAPP in cultured cells (23, 24), whereas others have not (25). Thus, one may speculate a direct or indirect “chaperone”-like effect of mutant PS or effects on intracellular vesicular trafficking to increase the susceptibility of βAPP to be cleaved at position 42.

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Several questions arise from these observations. The mechanistic effects of a given single amino acid substitution in mutant PS protein leading to AD are not yet understood. Previous data showing that either of the two known PS2 mutations, i.e. the Volga German mutation that substitutes Asn-141 for Ile (N141I) or the Italian mutation causing a Met-239 to Val (M239V) mutation, considerably increase the percentage of secreted Aβ42 (17, 18, 26), suggesting that a single amino acid substitution on PS2 may lead to a significant change in the protein folding and/or interaction with other proteins of PS2 compared with those with wild-type PS2. To gain insights into the nature of pathogenic effects caused by PS2 mutations, we first examined whether a mutant PS2 molecule harboring both the N141I and M239V mutations would increase the overproduction of Aβ42, compared with singly mutated PS2 with one or the other of these mutations.

PS1 and PS2 have been shown to undergo endoproteolytic cleavage that yields a long NH₂-terminal fragment (NTF) and a short COOH-terminal fragment (CTF) spanning the membrane 6 and 2 times, respectively (18, 27). These fragments are the predominant forms of PS1 or PS2 in cultured cells or brain tissues that do not overexpress PS (27). However, the relationship between cleavage and function of PS is not well understood. Next we sought to examine if the NTF or CTF forms of mutant PS2 alone are capable of promoting the secretion of Aβ42. To this end, we expressed partially truncated forms of PS2 (eliminating the COOH-terminal third of PS2 while retaining the endoproteolytic NTF) harboring a N141I mutation, as well as CTFs of PS2 in cultured cells and examined the COOH-terminal properties of Aβ secreted from these cells.

**EXPERIMENTAL PROCEDURES**

**Construction of Expression Plasmids**—A full-length cDNA encoding wild-type (WT) human PS2 was obtained by PCR from a normal human cDNA library, and the N141I or M239V PS2 mutations were introduced by the du-template method as described previously (18). WT as well as mutant (mt) PS2 cDNAs were subcloned into pBlueScript, and the coding region was then subcloned into a mammalian expression vector pcDNA3. The N141I/M239V double mutation was introduced by digesting the N141I mt PS2 cDNA in pBluescript Plasmid and then inserting the resultant ~0.5-kilobase pair product into the BamHI-BstXI sites of M239V mt PS2 in pcDNA3.

cDNAs encoding COOH-terminally truncated WT or N141I mt PS2 (i.e. PS2/270stop, PS2/303stop, and PS2/388stop) were generated by PCR using Pfu polymerase (Stratagene), and the following oligonucleotides were used as PCR primers: 5'CCGGATCCACCATGGCGAAGCTGGAC-3' for PS2/270stop, 5'GCCGATCCACCATGGGGCCTCTGAGA-3' for PS2/388stop, and 5'AGCTCGAGCTACCAACCGTCCA-3' for PS2/303stop, and 5'AGCTCGAGCTACTGTTGGTTCCAGC-3' for PS2/388stop as antisense primers, respectively. These primers were incubated with WT or N141I mt PS2 cDNAs in pcDNA3. cDNAs encoding varying lengths of the coding region was then subcloned into a mammalian expression vector and stable N2a cell lines were generated by transfecting the cDNAs in pcDNA3 vector using a mammalian transfection kit (Stratagene) or LipofectAMINE (Life Technologies, Inc.) and selection in Dulbecco's modified Eagle's modified Eagle's medium supplemented with 10% fetal calf serum and penicillin/streptomycin at 37 °C in 5% CO₂ atmosphere as described (18). Transient expression in COS-1 cells was performed by the DEAE-dextran method, and stable N2a cell lines were generated by transfecting the cDNAs in pcDNA3 vector using a mammalian transfection kit (Stratagene) or LipofectAMINE (Life Technologies, Inc.) and selection in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and penicillin/streptomycin at 37 °C in 5% CO₂ atmosphere as described (18). Transient expression in COS-1 cells was performed by the DEAE-dextran method, and stable N2a cell lines were generated by transfecting the cDNAs in pcDNA3 vector using a mammalian transfection kit (Stratagene) or LipofectAMINE (Life Technologies, Inc.) and selection in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and penicillin/streptomycin at 37 °C in 5% CO₂ atmosphere as described (18). Transient expression in COS-1 cells was performed by the DEAE-dextran method, and stable N2a cell lines were generated by transfecting the cDNAs in pcDNA3 vector using a mammalian transfection kit (Stratagene) or LipofectAMINE (Life Technologies, Inc.) and selection in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and penicillin/streptomycin at 37 °C in 5% CO₂ atmosphere as described (18).

**Cell Culture and Transfection**—Monkey COS-1 cells or mouse neuro2A (N2a) cells were maintained in Dulbecco's modified Eagle's modified Eagle's medium supplemented with 10% fetal calf serum and penicillin/streptomycin at 37 °C in 5% CO₂ atmosphere as described (18). Transient expression in COS-1 cells was performed by the DEAE-dextran method, and stable N2a cell lines were generated by transfecting the cDNAs in pcDNA3 vector using a mammalian transfection kit (Stratagene) or LipofectAMINE (Life Technologies, Inc.) and selection in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and penicillin/streptomycin at 37 °C in 5% CO₂ atmosphere as described (18). Transient expression in COS-1 cells was performed by the DEAE-dextran method, and stable N2a cell lines were generated by transfecting the cDNAs in pcDNA3 vector using a mammalian transfection kit (Stratagene) or LipofectAMINE (Life Technologies, Inc.) and selection in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and penicillin/streptomycin at 37 °C in 5% CO₂ atmosphere as described (18). Transient expression in COS-1 cells was performed by the DEAE-dextran method, and stable N2a cell lines were generated by transfecting the cDNAs in pcDNA3 vector using a mammalian transfection kit (Stratagene) or LipofectAMINE (Life Technologies, Inc.) and selection in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and penicillin/streptomycin at 37 °C in 5% CO₂ atmosphere as described (18). Transient expression in COS-1 cells was performed by the DEAE-dextran method, and stable N2a cell lines were generated by transfecting the cDNAs in pcDNA3 vector using a mammalian transfection kit (Stratagene) or LipofectAMINE (Life Technologies, Inc.) and selection in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and penicillin/streptomycin at 37 °C in 5% CO₂ atmosphere as described (18). Transient expression in COS-1 cells was performed by the DEAE-dextran method, and stable N2a cell lines were generated by transfecting the cDNAs in pcDNA3 vector using a mammalian transfection kit (Stratagene) or LipofectAMINE (Life Technologies, Inc.) and selection in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and penicillin/streptomycin at 37 °C in 5% CO₂ atmosphere as described (18). Transient expression in COS-1 cells was performed by the DEAE-dextran method, and stable N2a cell lines were generated by transfecting the cDNAs in pcDNA3 vector using a mammalian transfection kit (Stratagene) or LipofectAMINE (Life Technologies, Inc.) and selection in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and penicillin/streptomycin at 37 °C in 5% CO₂ atmosphere as described (18).
inhibited by a caspase-3 inhibitor, DEVD-CHO. In addition, a small amount of a 23-kDa CTF was detected. In N2a cells, 50–55-kDa fl PS2 (Fig. 2, arrow), 35-kDa NTF (Fig. 2, closed arrowhead), as well as a 23-kDa CTF (Fig. 2, open arrowhead) were detected. However, the amounts or ratios of NH_2- and COOH-terminal fragments were similar in M239V or N141I/M239V double mt PS2 compared with N141I mt or WT PS2 both in COS-1 and N2a cells (Fig. 2).

We then quantitated the levels of Aβ40 and Aβ42 secreted from cells transfected with these mt PS2 cDNAs. The percentages of Aβ42 secreted from COS-1 cells doubly transfected with bAPP C100 and N141I or M239V mt PS2 were elevated to almost similar levels (30%) by 2.2-fold compared with those from cells with WT PS2 and bAPP C100 (average of Aβ42, 13.8%), and the absolute levels of secreted Aβ42 were increased by 2.3 (N141I) and 1.7 (M239V) times, respectively, compared with WT PS2. When COS-1 cells were transfected with N141I/M239V double mutant PS2 and bAPP together with mt PS2 (data not shown).

We then examined the secretion of Aβ from stably transfected N2a cell lines expressing WT, N141I, M239V, or N141I/M239V mt PS2. As described previously (18), N2a cells expressing N141I mt PS2 secreted considerably increased amounts or percentages of Aβ42 (71.6% of total Aβ and 4.2 times compared with those with WT PS2: 17.0%). N2a cells expressing M239V mt PS2 also secreted significantly increased levels (3.4 times compared with those with WT PS2) or percentage (mean, 58.3%) of Aβ42. However, the secretion of Aβ42 from cells expressing N141I/M239V double mutant PS2 were again similar to those with either of the single PS2 mutations in terms of the absolute levels (4.1 times compared with those with WT PS2) or percentages (mean, 59.3%) (Fig. 3B). The expression of endogenous bAPP was at similar levels between different N2a cell lines (data not shown).

**Characterization of Truncated Forms of WT or N141I mt PS2 Proteins Expressed in Cultured Cells and Their Subcellular Localization**—To gain insights into the biological significance
Fragment Forms of PS2 and Aβ42 Overproduction

Fig. 3. Secreted Aβ40 and Aβ42 from cells expressing WT or single or double mutant PS2 genes. Levels of Aβ40–40 and Aβ40–42 secreted from COS-1 cells doubly transfected with βAPP C100 and PS2 genes (A) or N2a cells stably transfected with PS2 genes or an empty vector (B) quantitated by two-site ELISAs are shown. Mean values ± S.E. in four independent experiments in A and two independent experiments in B are shown. Transfected PS2 cDNAs are indicated below the columns; PS2WT, wild-type PS2; PS2N141I/M239V, N141I mutant PS2; PS2N141I, M239V mutant PS2; and PS2N141I/M239V, N141I and M239V double mutant PS2.

Fig. 4. Expression and metabolism of PS2 in transiently transfected COS-1 cells with cDNAs encoding truncated PS2. A, Western blot analysis of expression of WT or mt PS2 NTFs in transiently transfected COS-1 cells. Cell lysates (10 μg of protein) from COS-1 cells transfected with an empty pcDNA3 vector or with WT or N141I mt fl, WT or N141I mt 270stop, WT or N141I mt 303stop, and WT or N141I mt 388stop PS2 cDNAs were fractionated by SDS-PAGE and analyzed by immunoblotting with anti-G2N2 antibody. The positions of fl PS2 and NTFs are marked by arrows and arrowheads, respectively. B, Western blot analysis of expression of PS2 CTFs in transiently transfected COS-1 cells. Cell lysates (10 μg of protein) from COS-1 cells transfected with an empty pcDNA3 vector or with WT fl, 271ctf, 304ctf, or 344ctf cDNAs were fractionated by SDS-PAGE and analyzed by immunoblotting with anti-G2L or anti-PS2C2 antibodies. The positions of fl PS2, 23-kDa standard CTF, and 19-kDa alternative CTF are marked by arrows, arrowheads, and asterisks, respectively. The names of the transfected cDNA constructs are indicated at the top of each lane. Molecular mass standards are shown in kilodaltons.

of endoproteolytic processing of PS2 and especially to examine whether the NTF or CTF of PS2 is biologically active, we then expressed truncated forms of WT or N141I mt PS2 in cultured cells and characterized their metabolism and subcellular localization. Two categories of cDNAs encoding truncated PS2 were used (see Fig. 1): (i) NTF constructs (WT or N141I mt) ending at residues 270, 303, or 388 (designated PS2/270stop, PS2/303stop, and PS2/388stop), retaining the NH2-terminal 6 (or 7) transmembrane (TM) domains and shorter than, or longer than the predicted size of native NTF, respectively (33, 34); accordingly, PS2/388stop retains the entire length of the loop region as well as the 7th TM domain of PS2; (ii) CTF constructs starting at residues 271, 304, or 344 (designated PS2/271ctf, PS2/304ctf, and PS2/344ctf), longer than, close to, or shorter than the predicted size of native CTF, respectively. PS2/344ctf is close to the size of the “ALG-3” fragment, which was found to inhibit cellular apoptosis (35).

In COS-1 cells, PS2/270stop, PS2/303stop, and PS2/388stop (WT or mt) were expressed as doublets migrating at 30–33, 32–35, and 45–50 kDa, respectively (Fig. 4A). PS2/271ctf, PS2/304ctf, and PS2/344ctf were expressed as 27–, 23–, and 16-kDa fragments, respectively (Fig. 4B). These polypeptides migrated at slightly slower positions than those estimated from their predicted sizes (PS2/270stop, 30.6 kDa; PS2/303stop, 37.2 kDa; PS2/388stop, 43.5 kDa; PS2/271ctf, 19.6 kDa; PS2/304ctf, 15.9 kDa; and PS2/344ctf, 11.3 kDa). In addition to the full-length transfected proteins, PS2/388stop yielded 35–40-kDa doublet proteins that were equivalent in size to the NTFs cleaved from fl PS2 (Fig. 4A, arrowhead); PS2/271ctf (Fig. 4B) also yielded proteolytic fragments of 23 (arrowhead) and 19 kDa (asterisk) in size, and the 19-kDa CTF was also observed with PS2/304ctf (Fig. 4B, asterisk). Immunopositive bands at higher molecular weight ranges relative to these polypeptides (Fig. 4, A and B) would presumably represent dimeric forms and/or aggregates of these proteins.

In stable N2a cells, the expression patterns of PS2 derivatives were essentially similar to those in COS-1 cells with some differences (Fig. 5). Notably, 45–50-kDa polypeptides corresponding to PS2/388stop were barely processed to form 35-kDa NTF (Fig. 5A) that was present in cells with fl PS2 (Fig. 5A, arrowhead). PS2/271ctf (Fig. 5B) and PS2/304ctf (Fig. 5B) did not produce proteolytic fragments of smaller sizes. The patterns of expression of endogenous βAPP were almost similar between these cell lines (data not shown).

Next we examined the subcellular localization of the PS2 NTFs or CTFs in COS-1 cells by immunofluorescence microscopy. Remarkably, all constructs encoding NTFs and CTFs of WT or N141I mt types of PS2 showed similar distribution in a fine meshlike pattern throughout the cytoplasm as well as dense immunostaining in the perikaryal areas, which corresponded to those with BiP, a marker for endoplasmic reticulum (Fig. 6). N2a stable cells also showed similar patterns of ER localization of PS2 derivatives (data not shown).

Characterization of Aβ Secreted from Cells Expressing Truncated Forms of WT or N141I mt PS2—We then quantitated the levels and percentages of Aβ40 and Aβ42 secreted from cells expressing truncated forms of N141I mt or WT PS2. In COS-1 cells doubly transfected with βAPP C100 and each of the three types of truncated mt PS2, the Aβ42 in total Aβ was −10% in all, and they were similar to those in cells with corresponding forms of truncated WT PS2, whereas Aβ42 comprised 22.3% of total Aβ in cells expressing fl mt PS2, which was 1.7 times relative to that in cells expressing fl WT PS2 (13.5%). However,
A

anti-G2N2

B

anti-G2L

FIG. 5. Expression and metabolism of PS2 in stably transfected N2a cells with cDNAs encoding truncated PS2. Western blot analysis of expression of WT or mt PS2 derivatives in stably transfected N2a cells is shown. Cell lysates (20 µg of protein) from N2a cells transfected with an empty pcDNA3 vector or with WT or N141I mt fl, WT or N141I mt 270stop, WT or N141I mt 303stop, WT or N141I mt 388stop, and 271ctf or 304ctf PS2 cDNAs were fractionated by SDS-PAGE and analyzed by immunoblotting with anti-G2N2 (A) or anti-G2L (B) antibodies. The positions of fl PS2 and NTFs (in A) or CTFs (in B) are marked by arrows and arrowheads, respectively. Note that PS2/303stop comigrates with the 35-kDa NTF in A and that PS2/304ctf comigrates with the 33-kDa standard CTF in B, respectively. The names of the transfected cDNA constructs are indicated at the top of each lane. Molecular mass standards are shown in kilodaltons.

FIG. 6. Immunofluorescence localization of truncated PS2 derivatives expressed in COS-1 cells. COS-1 cells transfected with WT fl (A and B), N141I mt fl PS2 (C), WT 270stop (D), WT 303stop (E), WT 388stop (F), N141I mt 270stop (G), N141I mt 303stop (H), N141I mt 388stop (I), 271ctf (J), 304ctf (K), or 344ctf (L) cDNAs were immunostained with appropriate anti-PS2 antibodies (A and C–L; the primary antibodies used are indicated in each panel) or doubly with an anti-BiP monoclonal antibody (B) and observed with a confocal microscope after labeling with fluorescein-conjugated anti-rabbit IgG secondary antibody (in A and B, together with Texas Red-conjugated anti-mouse IgG antibody). Scale bar, 10 µm.

the total levels of Aβ were increased by ~2-fold in cells with truncated mt PS2 compared with those with truncated WT PS2. When CTFs of PS2 were transfected together with βAPP C100, the Aβ42 ranged between 11.9 and 14.6%, which also was similar to cells with fl WT PS2 (Fig. 7A). Similar results were obtained in COS-1 cells doubly transfected with WT or Swedish-type mutant (i.e., 595/596 KM-NL) βAPP together with these PS2 derivatives (data not shown).

In N2a cells stably expressing three types of truncated forms of N141I mt PS2, the percentage of Aβ42 that comprised the total Aβ ranged between 14.2 and 18.0%, which was similar to those in cells with truncated (12.3–16.5%) or fl WT PS2 (20.5%), and the absolute amounts of secreted Aβ were at similar levels between cells expressing truncated WT or mt PS2 (Fig. 7B). This was in sharp contrast to the marked increase in the percentage or level of Aβ42 from cells with fl mt PS2 (52.1%). The levels as well as percentages of Aβ42 secreted from N2a cells expressing PS2 CTFs (10.1–14.2%) also were similar to those with fl WT PS2 or mock-transfected cells (Fig. 7B).

To examine if coexpression of mt PS2 NTF together with CTF reconstitutes overproduction of Aβ42, we transiently transfected PS2/304ctf in N2a cells stably expressing WT or mt PS2/303stop. Upon co-transfection of PS2/304ctf, the total levels of secreted Aβ were decreased by ~50% both in WT and mt PS2/303stop stable cells relative to those in mock-transfected cells, whereas the Aβ42 remained unchanged both in cells expressing WT (9.7% in double transfection versus 11.3% in mock transfection) and mt (11.1% in double transfection versus 10.3% in mock transfection) PS2/303stop (Fig. 7C).

DISCUSSION

In this study, we have clearly shown that (i) full-length PS2 harboring both N141I and M239V mutations in the same polypeptide induced overproduction of Aβ42 at similar levels to those in cells expressing PS2 with one or the other of these PS2 mutations (i.e., N141I or M239V); (ii) NTFs or CTFs of PS2 expressed in cells predominantly localize in ER; and (iii) cells expressing partially truncated PS2 (eliminating the COOH-terminal third of PS2 while retaining the endoproteolytic NH2-terminal fragment) and harboring a N141I mutation, as well as cells expressing COOH-terminal fragments of PS2, did not overproduce Aβ42, and the levels or percentages of Aβ42 were comparable with those in cells that expressed full-length and wild-type PS2 as well as fragments thereof.

The nature of the structural or functional changes of the polypeptide membranes protein PS2 caused by the two known mutations is not fully understood at present. Regarding the N141I Volga German PS2 mutation, a PS1 mutation at the homologous site (N135D) was reported (36), and these homologous residues in PS1 and PS2 are located at the NH2-terminal flank (designated N-cap position) of the second transmembrane (TM2) domain, which is believed to be important in the accurate positioning of the transmembrane a-helix structure (37). Another PS2 mutation of the Italian type (M239V) is situated within the TM5 domain; a PS1 mutation linked to FAD at the homologous site (M233T) was also documented (38), and substitution of Met for Val was observed in multiple residues in the TM2 domain of PS1 (i.e., M1439V and M1146F) (8), suggesting that Met to Val substitution may cause some common structural changes in the TM domains of PS1 or PS2. Our observation that the N141I/M239V double mutation did not have additive effects on the increase in the levels or percentages of
secreted Aβ42 suggests that Aβ42-promoting capacities of mt PS2 proteins reach the maximum level with a given single amino acid substitution (i.e. N141I or M239V). This contrasts with the recent observation that the Aβ42-promoting effects of M146L/L286V double mutant PS1 were additive (39) and also with the clinical observation that FAD patients with PS1 mutations develop AD at a uniformly early age, whereas the age of onset in Volga German families with the N141I PS2 mutation is variable and relatively late (40). The reason for these discrepancies is not clear at present. However, one should con-
sider the differences in the protein levels of endogenous PS1 versus PS2 in the brains of FAD patients (10, 18). For example, it may be that the changes in Aβ42-promoting effects of PS2 caused by a given single mutation per molecule is stronger than those with mt PS1, whereas the overall pathogenic effects of mt PS1 become more intense than those of mt PS2 because the total amount of PS1 proteins in neurons or brain tissues is higher than that of PS2.

NTFs or CTFs of PS2 of various sizes predominantly localized to ER. Recently, it has been shown that the NH2-terminal 166 residues, but not 138 residues, of PS2 are sufficient for the ER targeting, suggesting that the initial two transmembrane domains are necessary for ER localization (41). Our findings confirmed these observations with respect to the NTFs and further extended these data by showing that CTFs of PS2, including those corresponding to the COOH-terminal 103 amino acids (ALG-3) also localize to ER. Although the precise orientation of the membrane insertion of these CTFs is yet to be determined, the occurrence of “caspase-type” cleavage (32) of these CTFs in similar patterns to those observed in cells expressing fl PS2 suggests that the NH2-terminal portions (i.e. loop region of PS2) of these CTFs are properly oriented to the cytoplasmic side. Moreover, these CTFs harbor two transmembrane domains (i.e. TM7 and -8). Taken together, the COOH-terminal region may harbor other ER-targeting signal sequences besides those in the NH2-terminal region, or alternatively, the presence of multiple (i.e. more than two) TM domains, but not particular subregions, of PS2 may determine its ER localization.

The most unexpected, yet intriguing, finding in this study was that cells expressing COOH-terminally truncated N1411 mt PS2 that are equivalent to or longer than the endoproteolytic NH2-terminal fragment did not overproduce Aβ42. This was surprising because most of the PS proteins in native cells or tissues (including brains) exist as NTF and CTF forms, and the NTFs contain six of the eight TM domains of PS molecules. Recently, it has been suggested that the levels of PS within cells are strictly regulated by competition for limiting cellular factors (29). Moreover, it was shown that NTF and CTF of PS1 or PS2 remain noncovalently bound to each other after cleavage forming a very stable complex (25, 42, 43) and that they may form a 100–150-kDa molecular mass complex (43). Our finding that NTF of mt PS2 or CTF alone does not promote Aβ42 overproduction supports the notion that the stable complex forms of PS NTF and CTF constitute the functional units under biological as well as pathological conditions. An alternative possibility would be that the nascent, full-length form of PS NTF and CTF constitute the functional units of PS. An alternative possibility would be that the nascent, full-length form of PS NTF and CTF constitute the functional units of PS. An alternative possibility would be that the nascent, full-length form of PS NTF and CTF constitute the functional units of PS. An alternative possibility would be that the nascent, full-length form of PS NTF and CTF constitute the functional units of PS. An alternative possibility would be that the nascent, full-length form of PS NTF and CTF constitute the functional units of PS. An alternative possibility would be that the nascent, full-length form of PS NTF and CTF constitute the functional units of PS. An alternative possibility would be that the nascent, full-length form of PS NTF and CTF constitute the functional units of PS. An alternative possibility would be that the nascent, full-length form of PS NTF and CTF constitute the functional units of PS. An alternative possibility would be that the nascent, full-length form of PS NTF and CTF constitute the functional units of PS. An alternative possibility would be that the nascent, full-length form of PS NTF and CTF constitute the functional units of PS. An alternative possibility would be that the nascent, full-length form of PS NTF and CTF constitute the functional units of PS. An alternative possibility would be that the nascent, full-length form of PS NTF and CTF constitute the functional units of PS. An alternative possibility would be that the nascent, full-length form of PS NTF and CTF constitute the functional units of PS. An alternative possibility would be that the nascent, full-length form of PS NTF and CTF constitute the functional units of PS. An alternative possibility would be that the nascent, full-length form of PS NTF and CTF constitute the functional units of PS. An alternative possibility would be that the nascent, full-length form of PS NTF and CTF constitute the functional units of PS. An alternative possibility would be that the nascent, full-length form of PS NTF and CTF constitute the functional units of PS. An alternative possibility would be that the nascent, full-length form of PS NTF and CTF constitute the functional units of PS. An alternative possibility would be that the nascent, full-length form of PS NTF and CTF constitute the functional units of PS. An alternative possibility would be that the nascent, full-length form of PS NTF and CTF constitute the functional units of P
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