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Familial Alzheimer's Disease Mutations in Presenilin 1 Do Not Alter Levels of the Secreted Amyloid- β Protein Precursor Generated by β -Secretase Cleavage

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Abstract

Alzheimer's disease (AD) is an insidious and progressive disease with a genetically complex and heterogeneous etiology. More than 200 fully penetrant mutations in the amyloid β -protein precursor (APP), presenilin 1 (or *PSEN1*), and presenilin 2 (*PSEN2*) have been linked to early-onset familial AD (FAD). 177 *PSEN1* FAD mutations have been identified so far and account for more than ~80% of all FAD mutations. All *PSEN1* FAD mutations can increase the A β 42:A β 40 ratio with seemingly different and incompletely understood mechanisms. A recent study has shown that the 286 amino acid N-terminal fragment of APP (N-APP), a proteolytic product of β -secretase-derived secreted form of APP (sAPP β), could bind the death receptor, DR6, and lead to neurodegeneration. Here we asked whether *PSEN1* FAD mutations lead to neurodegeneration by modulating sAPP β levels. All four different *PSEN1* FAD mutations tested (in three mammalian cell lines) did not alter sAPP β levels. Therefore PS1 mutations do not appear to contribute to AD pathogenesis via altered production of sAPP β .

Keywords

Alzheimer's disease; FAD mutation; APP; *PSEN1*; N-APP; sAPP β

INTRODUCTION

AD is a devastating neurodegenerative disease and the primary cause of dementia in the elderly. The genetic etiology of AD is complex and heterogeneous based on age of onset. Early-onset AD (<60 years; 5–10% cases) is often familial (FAD) with autosomal dominant and fully penetrant inheritance [1–3]. This form can be caused by any of more than 200 mutations in three genes: APP (31 mutations; duplic.), presenilin 1 (*PSEN1*; 177 mutations) and presenilin 2 (*PSEN2*; 14 mutations) (Alzheimer Disease & Frontotemporal Dementia Mutation Database; <http://www.molgen.ua.ac.be/ADMutations>; [1–3]). Most (90–95%) AD cases are late-onset, with the ϵ 4 variant of the gene encoding apolipoprotein E (*APOE*) known to confer increased risk with partial penetrance. More than 500 genes have been tested as late-onset AD candidate genes, of which over two dozen yield positive evidence for

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers Web site along with the published article.

association based on meta-analyses (<http://alzgene.org>; [1]). Considerable genetic, biochemical, molecular, and pathological evidence suggests that the excessive accumulation of the 4 kDa peptide, known as A β , is the primary pathological event leading to AD[2, 4]. A β is produced via serial cleavage of APP by β -secretase (BACE) and γ -secretase (Fig. 1).

The initial cleavage of APP can occur through α -, or β -secretase (Fig. 1). α -secretase cleavage produces sAPP α and the carboxy-terminal fragment, α -CTF (or C83); β -secretase cleavage produces the secreted polypeptide, sAPP β , and the β -carboxy-terminal fragment, β -CTF (or C99). C83 and C99 can be further cleaved by γ -secretase, which produces P3 or A β , respectively. A β can be 37–43 amino acids long depending on the γ -secretase cleavage site [5]. While A β 42 and A β 40 are the two primary A β species, A β 42 is more prevalent than A β 40 in amyloid plaques. Most mutations in *APP*, *PSEN1*, and *PSEN2* increase the ratio of A β 42:A β 40, which drives the aggregation of A β into neurotoxic oligomeric assemblies [1, 2]. Since this molecular phenotype is driven by β -secretase, it is believed that most early-onset FAD mutations drive AD pathogenesis downstream of β -secretase activity. In a recent study, Nikolaev et al identified a novel APP proteolytic molecule, N-APP, which has been proposed to contribute to AD pathogenesis[6]. N-APP is proteolytically derived from sAPP β following trophic-factor deprivation of primary neuronal cultures. N-APP contains the N-terminal 286 amino acids in APP and is ~35kDa (N-APP in Fig. 1). The resulting carboxy-terminal fragment is ~55kDa (sAPP55 shown in Fig. 1).

One of the fundamental processes in AD pathology has been proven to include caspase activation and apoptosis [6, 7], in which PS1 FAD mutations play important roles[7–10]. FAD mutant forms of PS1 have been proposed as pro-apoptotic effectors, e.g. PS1- Δ 9 [8]. N-APP, derived from sAPP, was shown to bind DR6 and trigger neurodegeneration through caspase 6 in axons and caspase 3 in cell bodies[6]. These data suggest that neurodegeneration in AD may be driven by β -secretase cleavage of APP, upstream of γ -secretase cleavage of APP. Thus, we set out to test whether FAD mutations in *PSEN1* may also drive AD neurodegeneration via effects on β -secretase cleavage of APP and altered sAPP β levels, upstream of N-APP.

METHODS

Cell Culture

H4 human neuroglioma cell line stably over-expressing human APP751 (H4-APP751) have been reported previously [11, 12]. Murine neuroblastoma B104 cells were transfected with human APP751 plasmid which was driven by the CMV promotor in the pORFex13 vector. One single colony was selected, amplified and identified to stably over-express APP751 (B104-APP751). Chinese hamster ovary (CHO) cells stably over-expressing human APP751 (CHO-APP751) were previously described [13]. These cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, 100 μ g/ml streptomycin, and 200ug/ml G418.

Plasmids, Chemicals and Antibodies

The PS1 wild type and mutants were constructed in the vector pcDNA3.1 and have been previously reported[14]. The APP C-terminal antibody (targeting the last 19 amino acids of APP751, APP750 or APP695; A8717; 1:1000) was purchased from Sigma. The sAPP β antibody (targeting ISEVKM, the C-terminus of human sAPP β wide type, 2ug/ml or 1:50) was from IBL. β -Actin antibody (1:10,000) was purchased from Sigma. The HRP-conjugated secondary antibodies (anti-mouse and anti-rabbit) (1:10,000) were purchased from Pierce.

Transfection

Transfections were done using the 96-well nucleofection shuttle system from Lonza (previously Amaxa). 150K cells were mixed with 0.5ug plasmid DNA, and resuspended in 20ul amaxa transfection solution SF. Program DS137 (for neuroblastoma cell line) was used for cell transfection. The transfected cells were placed in a 24-well plate containing 500ul cell culture medium. The cells were harvested 48 h after transfection.

Cell Lysis and Protein Amount Quantification

Cells were lysed in the M-PER Mammalian Protein Extraction Reagent (Thermoscientific) with 1X Halt protease inhibitor cocktail (Thermoscientific). The lysates were collected, centrifuged at 13,000 rpm for 20 minutes. Pellets were discarded and supernatants were transferred into a new Eppendorf tube. Total proteins were quantified by the BCA protein assay kit (Pierce).

Western Blotting Analysis

Western blotting analysis was carried out by the method described previously [15, 16]. Briefly, after centrifugation and protein concentration measurement, an equal amount of protein was applied to electrophoresis followed by membrane transfer, antibody incubation and signal development. Signals from sAPP β , full length APP (APP-FL) and β -actin were quantified[15, 16].

Data Analysis

sAPP β levels from each sample were normalized to the BCA values from the same cell lysates. To account for any differences in loading, APP-FL levels were normalized to the β -actin values from the same lane. Then normalized sAPP β values were divided by normalized APP-FL levels from each sample. The ratios of sAPP β /APP-FL from PS1 mutants were normalized to PS1 wide type, and were demonstrated as means $\pm$ SEM from four independent experiments. We used a two-tailed t test to compare the difference between the experimental groups. P values < 0.05 were considered statistically significant.

RESULTS

We tested whether four previously reported PS1 FAD mutations (A246E, M146L, M146V, and Δ 9) alter sAPP β levels and the ratio of sAPP β /APP-full length (FL)[10]. First, we tested the effects of these mutations in human neuroglioma H4 cells stably overexpressing human APP751 (H4-APP751) [11, 12]. These cells were transiently transfected with each of the four FAD mutant PS1 plasmids. Control cells were transfected with the empty vector pcDNA3.1 plasmid, as well as pcDNA3.1 with wild type-PS1 (PS1-WT). Quantitative Western blotting analysis was performed on the sAPP β values for each sample, which were further normalized to their corresponding protein concentration represented by the BCA levels. Full-length APP levels were also quantitated and normalized to β -actin levels from the same sample. The ratio of sAPP β /APP was calculated for each sample, and comparisons were made for each PS1 FAD mutant versus PS1-WT (Figs. **2A**, **2B**). The sAPP β from PS1-WT was set as 100% or 1, and PS1 FAD mutants, as well as empty vector, were normalized to PS1-WT. The same analytic protocols were also applied to the calculation of APP-FL and sAPP β /APP-FL. We observed no significant differences for sAPP β /APP-FL among the PS1-WT and mutant FAD PS1 cells ($p > 0.05$) (Fig. **2C**).

Because PS1 metabolism can exhibit cell-specific differences[17, 18], we next asked whether PS1 FAD mutations could alter sAPP β and/or sAPP β /APP levels in other cell lines. For this purpose, we tested B104 rat neuroblastoma cells over-expressing human APP751 (B104-APP751). We transiently and individually transfected the cells with the same PS1

FAD mutant plasmids from the previous experiment and measured the sAPP β levels, the total APP levels and calculated the sAPP β /APP values (Figs. 3A, 3B). We found that no significant differences existed among the PS1-WT and PS1 FAD mutant cells ($p>0.05$) (Fig. 3C). Finally, we tested Chinese hamster ovary cells over-expressing human APP751 (CHO-APP751) using the same protocol described above. CHO-APP751 cells were respectively and transiently transfected with the four PS1 FAD mutant plasmids, the PS1-WT and the empty vector. We measured the sAPP β levels, the total APP levels and calculated the ratios of sAPP β /APP (Figs. 4A, 4B). We found no significant differences among the PS1-WT and PS1 FAD mutant cells ($p>0.05$) (Fig. 4C).

DISCUSSION

Most mutations in *APP*, *PSEN1*, and *PSEN2* increase the ratio of A β 42:A β 40, which accelerates the oligomerization of A β into neurotoxic assemblies [1, 2]. This ratio depends on β -secretase cleavage, downstream of β -secretase activity. Recently, Nikolaev *et al.* suggested that N-APP, a secreted molecule proteolytically derived from sAPP β , contributes to AD pathogenesis[6]. Thus, we tested whether FAD mutations in *PSEN1* may also drive AD neurodegeneration by altering sAPP β levels, upstream of N-APP. Four different *PSEN1* FAD mutations had no effect on sAPP β levels in three different mammalian cell lines versus wild type *PSEN1*. Thus, *PSEN1* FAD mutations lead to AD pathogenesis through pathways other than sAPP β (and N-APP) production. Our results are in agreement with Gandy *et al.* (2001), which refuted a claim by Russo *et al.* (2001), that *PSEN1* mutants may alter β -secretase cleavage of APP [19, 20]. Here, we definitively show that the four *PSEN1* FAD mutations tested do not affect β -secretase cleavage of APP. As expected, all four *PSEN1* FAD mutations tested in our study increased the A β 42/A β 40 ratio (supplemental data) [5, 17, 18, 21, 22].

The results in the Nikolaev *et al.* study support the use of β -secretase inhibitors as AD therapeutics to prevent neurodegeneration of sensory axons and cell bodies[6]. On the other hand, stimulating γ -secretase has also been proposed as a potentially useful therapeutic strategy[23]. Although most *PSEN1* FAD mutations increase the ratio of A β 42/A β 40, the underlying pathogenetic mechanism is still not completely understood. In addition to increasing A β 42/A β 40 levels, PS1 FAD mutations have been shown to render cells more susceptible to caspase activation and apoptosis, however the roles of these processes in AD pathogenesis remain unclear [7–10]. We note that the interaction between N-APP and DR6 was not examined in this study; we show that levels of sAPP β , which gives rise to N-APP, were not affected by four *PSEN1* FAD mutations. Thus, our data argue against a role for sAPP β and its byproduct, N-APP, in AD pathogenesis driven by early-onset FAD mutations in *PSEN1*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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REFERENCES

1. Bertram L, Tanzi RE. Thirty years of Alzheimer's disease genetics: the implications of systematic meta-analyses. *Nat Rev Neurosci.* 2008; 9:768–778. [PubMed: 18802446]
2. Tanzi RE, Bertram L. Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. *Cell.* 2005; 120:545–555. [PubMed: 15734686]

3. Tanzi RE, Gusella JF, Watkins PC, Bruns GA, St George-Hyslop P, Van Keuren ML, et al. Amyloid beta protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus. *Science*. 1987; 235:880–884. [PubMed: 2949367]
4. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer' disease: progress and problems on the road to therapeutics. *Science*. 2002; 297:353–356. [PubMed: 12130773]
5. Page RM, Baumann K, Tomioka M, Perez-Revuelta BI, Fukumori A, Jacobsen H, et al. Generation of Abeta38 and Abeta42 is independently and differentially affected by familial Alzheimer disease-associated presenilin mutations and gamma-secretase modulation. *J Biol Chem*. 2008; 283:677–683. [PubMed: 17962197]
6. Nikolaev A, McLaughlin T, O'Leary DD, Tessier-Lavigne M. APP binds DR6 to trigger axon pruning and neuron death via distinct caspases. *Nature*. 2009; 457:981–989. [PubMed: 19225519]
7. Kim TW, Pettingell WH, Jung YK, Kovacs DM, Tanzi RE. Alternative cleavage of Alzheimer-associated presenilins during apoptosis by a caspase-3 family protease. *Science*. 1997; 277:373–376. [PubMed: 9219695]
8. Xie Z, Romano DM, Kovacs DM, Tanzi RE. Effects of RNA interference-mediated silencing of gamma-secretase complex components on cell sensitivity to caspase-3 activation. *J Biol Chem*. 2004; 279:34130–34137. [PubMed: 15184387]
9. Xie Z, Romano DM, Tanzi RE. Effects of RNAi-mediated silencing of PEN-2, APH-1a, and nicastrin on wild-type vs FAD mutant forms of presenilin 1. *J Mol Neurosci*. 2005; 25:67–77. [PubMed: 15781968]
10. Kovacs DM, Mancini R, Henderson J, Na SJ, Schmidt SD, Kim TW, et al. Staurosporine-induced activation of caspase-3 is potentiated by presenilin 1 familial Alzheimer's disease mutations in human neuroglioma cells. *J Neurochem*. 1999; 73:2278–2285. [PubMed: 10582585]
11. Xie Z, Dong Y, Maeda U, Xia W, Tanzi RE. RNA interference silencing of the adaptor molecules ShcC and Fe65 differentially affect amyloid precursor protein processing and Abeta generation. *J Biol Chem*. 2007; 282:4318–4325. [PubMed: 17170108]
12. Xie Z, Romano DM, Tanzi RE. RNA interference-mediated silencing of X11alpha and X11beta attenuates amyloid beta-protein levels via differential effects on beta-amyloid precursor protein processing. *J Biol Chem*. 2005; 280:15413–15421. [PubMed: 15699037]
13. Huttunen HJ, Guenette SY, Peach C, Greco C, Xia W, Kim DY, et al. HtrA2 regulates beta-amyloid precursor protein (APP) metabolism through endoplasmic reticulum-associated degradation. *J Biol Chem*. 2007; 282:28285–28295. [PubMed: 17684015]
14. Kimberly WT, LaVoie MJ, Ostaszewski BL, Ye W, Wolfe MS, Selkoe DJ. Gamma-secretase is a membrane protein complex comprised of presenilin, nicastrin, Aph-1, and Pen-2. *Proc Natl Acad Sci USA*. 2003; 100:6382–6387. [PubMed: 12740439]
15. Hiltunen M, Lu A, Thomas AV, Romano DM, Kim M, Jones PB, et al. Ubiquilin 1 modulates amyloid precursor protein trafficking and Abeta secretion. *J Biol Chem*. 2006; 281:32240–32253. [PubMed: 16945923]
16. Zhang C, Khandelwal PJ, Chakraborty R, Cuellar TL, Sarangi S, Patel SA, et al. An AICD-based Functional Screen to Identify APP Metabolism Regulators. *Mol Neurodegener*. 2007; 2:15. [PubMed: 17718916]
17. Xia W, Zhang J, Kholodenko D, Citron M, Podlisny MB, Teplow DB, et al. Enhanced production and oligomerization of the 42-residue amyloid beta-protein by Chinese hamster ovary cells stably expressing mutant presenilins. *J Biol Chem*. 1997; 272:7977–7982. [PubMed: 9065468]
18. Zhang J, Kang DE, Xia W, Okochi M, Mori H, Selkoe DJ, et al. Subcellular distribution and turnover of presenilins in transfected cells. *J Biol Chem*. 1998; 273:12436–12442. [PubMed: 9575200]
19. Gandy S, Naslund J, Nordstedt C. Alzheimer's disease. Molecular consequences of presenilin-1 mutation. *Nature*. 2001; 411:654–656. [PubMed: 11395757]
20. Russo C, Schettini G, Saido TC, Hulette C, Lippa C, Lannfelt L, et al. Presenilin-1 mutations in Alzheimer's disease. *Nature*. 2000; 405:531–532. [PubMed: 10850703]
21. Xia W, Ostaszewski BL, Kimberly WT, Rahmati T, Moore CL, Wolfe MS, et al. FAD mutations in presenilin-1 or amyloid precursor protein decrease the efficacy of a gamma-secretase inhibitor:

- evidence for direct involvement of PS1 in the gamma-secretase cleavage complex. *Neurobiol Dis.* 2000; 7:673–681. [PubMed: 11114265]
22. Xia W, Zhang J, Ostaszewski BL, Kimberly WT, Seubert P, Koo EH, et al. Presenilin 1 regulates the processing of beta-amyloid precursor protein C-terminal fragments and the generation of amyloid beta-protein in endoplasmic reticulum and Golgi. *Biochemistry.* 1998; 37:16465–16471. [PubMed: 9843412]
23. Zhang C, Saunders AJ. Therapeutic targeting of the alpha-secretase pathway to treat Alzheimer's disease. *Discov Med.* 2007; 7:113–117. [PubMed: 18093473]

Fig. (1).

Summary of the proteolytic processing of APP. The early-onset FAD gene *APP* encodes amyloid β -protein precursor, which generates $A\beta$ by the serial proteolytic cleavage through β -, and γ -secretase. β -secretase cleavage produces the secreted, ~90kDa polypeptide, sAPP β , and the β -carboxy-terminal fragment, β -CTF (or C99). sAPP β is the substrate of an unidentified secretase, which produces N-APP (containing the N-terminal 286 amino acids of APP; ~35kDa) and s-APP55 (~55kDa). C99 can be cleaved by γ -secretase and gives rise to $A\beta$ and AICD (APP intracellular domain). In contrast to this amyloidogenic process by β -, and γ -secretase, APP undergoes a different serial cleavage pathway which precludes $A\beta$ generation. This pathway is initiated by α -secretase and produces sAPP α and the carboxy-terminal fragment, α -CTF (or C83). C83 can be further cleaved by γ -secretase and produces P3 and AICD.

Fig. (2).

PS1 mutations did not alter the levels of sAPPβ, APP-FL and the ratio of sAPPβ/APP in H4-APP751 cells. **A.** H4-APP751 cells were respectively and transiently transfected with six different plasmids: 1. the empty vector pcDNA3.1; 2. PS1-wild type (WT); 3. PS1-Δ9; 4. PS1-M146L; 5. PS1-M146V; 6. PS1-A246E. Conditioned media and cell lysates were collected 48h post transfection. Conditioned media was applied to Western blotting analysis to calculate sAPPβ levels utilizing the sAPPβ antibody. **B.** Cell lysates were subjected to bicinchoninic acid (BCA) assays to calculate protein concentration, followed by Western blotting analysis. APP antibody (A8717) was used to measure the full length APP levels. β-Actin antibody was used to normalize the loading volume compared in each lane. **C.** The

levels of sAPP β , APP-FL and the ratio of sAPP β /APP-FL in each treatment. sAPP β levels were normalized to cell lysate protein levels, and APP-FL levels were normalized to β -actin levels from the same sample. Normalized sAPP β was divided by corresponding APP-FL levels for each sample to represent the ratio of sAPP β /APP-FL. PS1 FAD mutations and empty vector were normalized to PS1-WT which was set as 100% or 1. The data were consolidated and demonstrated as means $\pm$ SEM from four independent experiments. For the levels of sAPP β and APP-FL and the ratios of sAPP β /APP, there were no differences between the four PS1 FAD mutations and the PS1-WT ($p>0.05$).

Fig. (3).

PS1 mutations did not alter the levels of sAPPβ, APP-FL and the ratio of sAPPβ/APP in B104-APP751 cells. **A.** B104-APP751 cells were respectively and transiently transfected with six different plasmids: 1. the empty vector pcDNA3.1; 2. PS1-wild type (WT); 3. PS1-Δ9; 4. PS1-A246E; 5. PS1-M146L; 6. PS1-M146V. Conditioned media and cell lysates were collected 48h post transfection. Conditioned media was applied to Western blotting analysis to calculate sAPPβ levels utilizing the sAPPβ antibody. **B.** Cell lysates were subjected to bicinchoninic acid (BCA) assays to calculate protein concentration, followed by Western blotting analysis. APP antibody (A8717) was used to measure the full length APP levels. β-Actin antibody was used to normalize the loading volume compared in each lane. **C.** The

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Fig. (4).

PS1 mutations did not alter the levels of sAPP β , APP-FL and the ratio of sAPP β /APP in CHO-APP751 cells. **A.** CHO-APP751 cells were respectively and transiently transfected with six different plasmids: 1. the empty vector pcDNA3.1; 2. PS1-wild type (WT); 3. PS1- Δ 9; 4. PS1-A246E; 5. PS1-M146L; 6. PS1-M146V. Conditioned media and cell lysates were collected 48h post transfection. Conditioned media was applied to Western blotting analysis to calculate sAPP β levels utilizing the sAPP β antibody. **B.** Cell lysates were subjected to bicinchoninic acid (BCA) assays to calculate protein concentration, followed by Western blotting analysis. APP antibody (A8717) was used to measure the full length APP levels. β -Actin antibody was used to normalize the loading volume compared in each lane. **C.** The

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