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Monogenic determinants of familial Alzheimer's disease: presenilin-1 mutations

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Abstract. Presenilin-1 (*PS1*) mutations account for the greatest portion of early onset familial Alzheimer's disease (FAD) cases. The exact cellular function of *PS1* is not known. To date, *PS1* mutations have been shown to alter two potential biological roles of the protein, either of which could make neurons more susceptible to neurodegeneration. First, *PS1* mutations result in elevated Aβ42/Aβ40 ratios in plasma of FAD patients, in transgenic mice and in transfected cell lines. Aβ42 is the more hydrophobic and most neurotoxic form of the peptide. A common molecular event that has been associated with all of the known early onset FAD genes is the excessive production or accumulation of the Aβ peptide in the brain. *PS1* mutations have also been found to alter the Notch signalling pathway, but the mechanism by which this may affect neurodegeneration remains to be determined. Future studies will be needed to elucidate whether *PS1* mutations lead directly to neuronal dysfunction and degeneration or cause cell death by increasing Aβ42 generation and deposition.

Key words. PS1; presenilin-1; Alzheimer's disease; FAD; neurodegeneration; β-amyloid; mutations.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder of the central nervous system (CNS) that is characterized by global cognitive decline including the progressive loss of memory, orientation and reasoning. The brains of patients with AD contain abundant amounts of neurofibrillary tangles (NFT) and β-amyloid in the form of senile plaques and cerebral blood vessels. While the aetiological events that lead to AD remain unresolved, a large percentage of AD has been shown to be of genetic origin (for review see [refs 1, 2]) and is termed familial Alzheimer's disease (FAD). FAD is a genetically heterogeneous disorder that can be categorized according to age of onset using 60 years as the cut-off for 'early-onset' vs. 'late-onset' FAD. The gene defects responsible for early-onset (< 60 years) FAD are located on chromosomes 21, 14 and 1 and include the genes for amyloid β-protein precursor (*APP*), presenilin-1 (*PS1*) and presenilin-2 (*PS2*), respectively. The only well confirmed late-onset AD risk factor that has been identified is the *APOE* gene, located on chromosome 19 [3].

Mutations in the *APP* gene account for a very small proportion (< 1%) of all published cases of FAD [4] and no more than 2–3% of reported cases of early-onset FAD. The pathogenicity of these mutations has been strongly supported by the fact that they are virtually 100% penetrant in FAD kindreds where they occur in affected or at-risk individuals, but are absent in age-matched controls. Presenilin mutations, largely contained in the *PS1* gene, are responsible for approximately 50% of early onset FAD cases primarily with onset below the age of 50 [5]. With only one reported exception [6], the FAD mutations in the presenilinsappear to be 100% penetrant and are inherited as autosomal dominant 'causative' gene defects. A common

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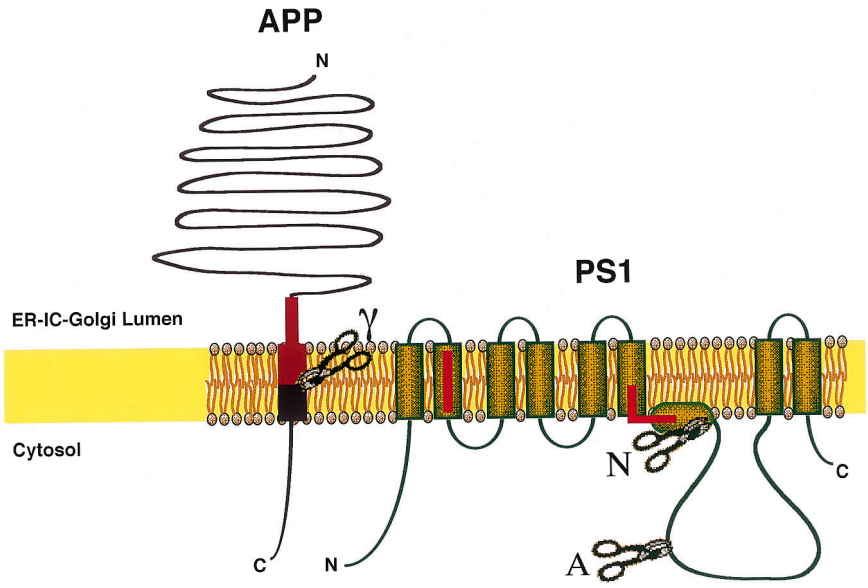

Figure 1. *PS1* and *APP* topology and cleavage in the ER-IC-Golgi. Transmembrane orientations of APPp and PS1p as they would be predicted to reside in the ER, intermediate compartment and Golgi, illustrating the possible PS1p domains affecting γ-secretase cleavage of APPp. Red segments in PS1p show the mutational clusters in transmembrane domain 2 and in hydrophylic loop 6. The dark red segment in APPp indicates the position of the Aβ domain. γ, γ-secretase cleavage; N, 'normal' cleavage; A, 'alternative' cleavage.

polymorphism in the *APOE* gene (*APOE* ε4) confers increased likelihood for late-onset FAD especially when inherited as two doses [3], but does not cause AD in a 'determinative' fashion.

Mutations in all four FAD genes have been shown to increase either the production or deposition of the Aβ peptide, the major component of β-amyloid in AD. The *APP* mutations are all missense mutations lying within or close to the domain encoding the Aβ peptide. All *APP* [7–10] and presenilin mutations tested appear to increase levels of total Aβ or the more amyloidogenic species, Aβ42, both in vitro and in vivo. Several studies have shown that the *APOE* ε4 allele confers an increase in the density of cerebral β-amyloid deposits. AD and Down's syndrome patients who are *APOE* ε4-positive exhibit increased amyloid burden in the brain, presumably due to increased aggregation or decreased clearance of Aβ [11]. Aβ deposition in *APOE* knockout mice is markedly decreased [12]. These data suggest that alterations in either the generation (mediated by *APP*, *PS1*, *PS2* alleles) or accumulation/clearance (mediated by *APOE* allele) of Aβ lead to β-amyloid deposition and that this may be the common pathogenic event associated with all four of the known FAD genes. Alternatively, since the apolipoprotein E protein (APOEp) normally plays a role in the storage and transport of cholesterol and since the *APOE* ε4 allele also predisposes to heart disease associated with high cholesterol levels, a potential role for cholesterol in promoting AD neuropathology cannot be ruled out.

The *PS1* gene and its expression

The *PS1* gene is located in the 14q24.3 region of chromosome 14 [13] and encodes for a 463-amino acid multispanning integral membrane protein with six to nine predicted transmembrane (TM) domains [13] (see fig. 1). At the amino acid level, the presenilin 1 protein (PS1p) is 67% identical to PS2p, also sharing the predicted overall serpentine structure [14, 15]. The greatest divergence between the two presenilins is found in the N-terminus and in the large hydrophilic loop (HL) towards the C-terminal part of each molecule. The overall similarity between the presenilins indicates that these two proteins would have similar functions. However, the two non-homologous regions in these molecules would appear to confer specificity of function for PS1p and PS2p. Presenilin homologues have been identified in mouse [13], *Drosophila* [16, 17] and *Caenorhabditis elegans* [18, 19]. The membrane topology of the *C. elegans* proteins, SEL-12p and HOP-1p,

is apparently similar to that of the presenilins. In *C. elegans*, the presenilin homologues appear to facilitate the function of the Notch receptor, which plays a role in cell-to-cell signalling during cellular differentiation in development [18]. How this facilitation occurs is not known, but may involve either a role for SEL-12p in downstream signalling or in the trafficking and localization of the Notch receptor.

To date, 45 different FAD mutations have been identified in *PS1* in over 75 families of various ethnic origins (for reviews, see [refs 5, 20]). All except one of the *PS1* mutations are missense mutations causing single amino acid changes. The exception is a mutation which destroys the splice acceptor site for exon 10 of *PS1*, resulting in an in-frame deletion of this exon and an amino acid substitution [21].

All FAD missense mutations identified to date occur in amino acids that are conserved between PS1p and PS2p. Although the known FAD mutations are distributed throughout the *PS1* gene, roughly 60% of all reported mutations cluster in exon 6, changing amino acids in predicted TM-2, and in exon 9, corresponding to HL-6 (see fig. 1). Interestingly, mutations in these clusters cause the most aggressive form of the disease with regard to age of onset. The mean age of onset of AD in all *PS1*-linked FAD families (including exon 6 and 9 mutations) is approximately 45 years, with a range of 28 to 62 years. Mutations in exon 6 reduce the mean age of onset to 40, and those in exon 9 to 43 years. In contrast, for all other combined mutations in *PS1*, the mean age of onset is considerably later (47 years). It is therefore likely that the conformation and/or function of the PS1p is particularly dependent on the amino acid sequences encoded by exons 6 and 9. It should also be noted that the two FAD mutation clusters in *PS1* occur in regions that are adjacent to the largest of the predicted luminal (HL-1/TM-2) and cytoplasmic (HL-6/TM-6) loops, suggesting that these loops may be critical to PS1p function.

The major *PS1* messenger RNA (mRNA) messages are 2.7 and 7.5 kb in size [13], with one reported alternatively spliced form missing four amino acids (VRSQ) at the 3′ end of exon 4 [22]. In human and rodent brains, *PS1* mRNA is detected primarily in neurons [23–26], although expression of *PS1* is ubiquitous [13]. In situ hybridization of *PS1* in human temporal lobe revealed highest expression in the neuronal laminae of the hippocampal formation, parahippocampal gyrus and choroid plexus [24, 26]. PS1p has also been immunohistochemically localized to neuronal somatodendritic compartments in human, monkey and mouse brains [27–30]. The expression patterns of *PS1* and *PS2* both at the mRNA and at the protein level are virtually identical [24, 14] and similar to that observed for *APP* 695 mRNA. The prevalent neuronal localization of the presenilins in brain suggests that neurons are most likely the cellular origin of neuropathological alterations in brains of FAD patients with *PS1* and *PS2* mutations. Although the presenilins are expressed more highly in brain areas that are vulnerable to AD neuropathology, the expression of *PS1* does not differ in AD vs. control brains [31].

PS1p

Subcellularly, both presenilins have been localized to the endoplasmic reticulum (ER) and the Golgi compartment in a variety of cell types including COS, H4, CHO and NT2N cells [24, 32–34]. Additionally, PS1p has been detected in dendrites of NT2N human neuronal cell lines expressing PS1p [33], and in human brain sections [27]. Meanwhile, plasma membrane staining was not reported for either of the presenilins by Kovacs et al. [24] or Cook et al. [33], but was suggested by Dewji and Singer [35]. In addition, Li et al. [36] have reported the nuclear localization of the presenilins. No gross abnormalities in the subcellular localization of mutant vs. wild-type PS1p have yet been reported.

PS1p has been shown to be phosphorylated by protein kinase C on its terminal end [37, 38], but is not N- or O-glycosylated [33]. Recent reports indicate that both presenilins most likely contain either six or eight TM domains, with both N-terminal and C-terminal domains and the largest hydrophylic loop (HL-6) oriented towards the cytoplasm [39, 40]. However, endogenous PS1p is cleaved into an ~28-kDa N-terminal and an ~19-kDa C-terminal fragment (CTF), and the full-length molecule of 45-50 kDa is generally not detectable endogenously [24, 41–44]. Full-length PS1p has also been found to be polyubiquitinated in vivo, and it is likely that a fraction of the full-length molecule undergoes degradation by the proteasomal pathway, as we have shown for PS2p [45]. This is supported by the observations that in transgenic mice expressing human PS1p, the N- and C-terminal fragments are produced to saturable levels (and in a strict 1:1 stoichiometry) and that the accumulation of fragments produced from the human transgene leads to a decrease in the endogenous mouse fragments [42, 46]. Interestingly, FAD mutations in the transgene have also been suggested to provoke an increase in the absolute amount of the accumulated endoproteolytic cleavage fragments [47].

The site of the endoproteolytic cleavage was identified in the domain encoded by exon 10 in the proximal portion of HL-6 [42], and more precisely after methionine 298, but also in a smaller amount before methionine 292 and valine 293 [48]. More recently, an 'alternative' cleavage product of *PS1* was identified to be the result of a more distal cleavage to the 'normal' endoprote-

olytic site in the domain encoded by exon 10 (see fig. 1). This alternative clip leads to the generation of a smaller, 14-kDa CTF [49, 50], and the cleavage site was shown to occur after aspartic acid 345, within a consensus sequence for caspase 3-type proteases. Consequently, this smaller CTF was detected either in cells undergoing apoptosis or in cells overexpressing high levels of PS1p [49]. Effects of *PS1* FAD mutations on the alternative cleavage remain to be studied, although it has been shown that in transfected cell lines the *PS2* N141I FAD mutation leads to a threefold increase in the production of the alternative PS2p CTF [49]. Generation of a 15-kDa PS1p CTF has also been reported to occur during neuronal differentiation [51, 52]. However, whether this fragment observed during neuronal differentiation is identical to the caspase-generated PS1p CTF is not yet clear.

Cleavage of PS1p by the family of cysteine proteases known as caspases was first shown by Kim et al. [49] employing specific caspase inhibitors. This was confirmed by successful competition of the PS1p caspase consensus sequence for the cleavage of a fluorogenic caspase substrate [50]. However, direct incubation of PS1p with recombinant caspase-3 (CPP-32) did not result in cleavage of PS1p, although PS2p was efficiently cleaved under the same conditions [50, 53]. Therefore, the identity of the exact enzyme cleaving the potential caspase site of PS1p has not been clearly determined. The role of PS1p in the apoptotic process itself is suggested by the fact that the L286V FAD mutation has been reported to sensitize neuronal cells to apoptosis induced by trophic factor withdrawal and by Aβ [54]. *PS2* has also been reported to increase sensitivity to apoptosis in PC-12 and HeLa cells, and the N141I mutation appears to accelerate this process [55–57]. On the other hand, transfection with an antisense *PS2* construct has been shown to block apoptosis. Perhaps unexpectedly, the C-terminal portion of mouse *PS2* has been reported to rescue cells undergoing Fas-mediated apoptosis [58]. Taken together, these findings suggest that both presenilins serve as cell death substrates and that *PS2* is likely to participate to the process of programmed cell death. However, the exact identity of the protease generating the alternative CTF in PS1p and the direct involvement of PS1p in the apoptotic process remain to be confirmed. The exact contribution of apoptosis to the pathogenesis of Alzheimer's disease is not yet clear (for reviews see refs 59–61). Future investigations aimed at determining the effects of FAD mutations on PS1p processing and degradation are likely to yield valuable clues regarding the mechanism by which *PS1* FAD mutations lead to AD neuropathological changes.

Biological and pathological effects of PS1 mutations

While the biological functions and physiological roles of the presenilins are not yet known, both have been proposed to possibly act as receptors, ion channels or molecules involved in protein processing or trafficking [13, 14, 24]. Mutations in the presenilins have so far been shown to alter two potential biological roles of these proteins, which could eventually foster neurodegeneration. The first concerns effects on Aβ deposition and on APPp metabolism, involving an increased $A\beta_{42}/A\beta_{40}$ ratio. The second involves the potential role of PS1p in the Notch signalling pathway, but how this relates to AD neuropathogenesis remains to be determined.

Increase in $A\beta_{42}/A\beta_{40}$ ratio

$A\beta_{42}$ levels (the amyloid peptide ending at amino acid 42 as opposed to amino acid 40) were shown to be increased in brains of AD patients, where they are the predominant species of the peptide to deposit in neuritic plaques as well as diffuse plaques (for review, see ref 5). Moreover, elevated $A\beta_{42}/A\beta_{40}$ ratios were found in association with the *APP* 717 FAD mutations [62, 63], indicating that increased $A\beta_{42}$ levels may be associated with the early-onset FAD pathology. Indeed, when plasma and fibroblasts of chromosome 14-linked FAD patients and at-risk family members were analysed, the $A\beta_{42}/A\beta_{40}$ ratio was found to be increased [64]. Similar increases in $A\beta_{42}/A\beta_{40}$ ratio have also been observed in transfected cell lines and transgenic mice expressing FAD mutant forms of *PS1* or *PS2* [65–69]. Finally, brains of FAD patients carrying mutations in *PS1* or *PS2* also predominantly show deposition of the $A\beta_{42/43}$ form of the peptide, although it is known that the amount of $A\beta_{42}$ synthesized is usually only about 10% of total Aβ [70–72]. Collectively, these data compellingly support the notion that the common molecular phenotype for virtually all of the known presenilin FAD mutations is the relatively increased production and deposition of the more amyloidogenic form of the Aβ peptide, $A\beta_{42}$.

The mechanism by which presenilin mutations may affect cleavage at the C-terminal end of Aβ (γ-secretase cleavage) is the subject of intense studies. Both $A\beta_{40}$ and $A\beta_{42}$ were initially thought to be generated exclusively through secretory endocytic pathways; however, the ER-Golgi localization of the presenilins and the selective increase in $A\beta_{42}$ as an effect of FAD mutations have raised the possibility that the two forms of Aβ may be produced in different subcellular compartments, perhaps involving distinct proteases. Indeed, three separate studies have recently shown

that $A\beta_{42}$ is generated in the ER [73–75]. Moreover, the production of $A\beta_{40}$, but much less of $A\beta_{42}$, was found to be differentially inhibited by a calpain inhibitor compound [76], suggesting that either the two forms of $A\beta$ are the product of two distinct γ-secretases, or that one single enzyme has different affinities for the 40–41 and the 42–43 peptide bonds perhaps as a consequence of its subcellular localization. Direct interaction between PS1p or PS2p and immature forms of APPp has also been demonstrated [77, 78], indicating a possible mechanism by which presenilin FAD mutations could directly affect APPp processing in the ER-Golgi compartments.

Notch pathway/inability to rescue a *sel-12*-defective phenotype

SEL-12p, a *C. elegans* protein functioning downstream from a Notch family receptor (LIN-12p), exhibits roughly 50% identity to the presenilins [18], raising the possibility that the presenilins are involved in the Notch signalling pathway during or after neural development. Indeed, it was found that PS1, PS2 and another *C. elegans* presenilin homologue, *HOP-1*, can rescue the *C. elegans sel-12* mutant phenotype [19, 79, 80], which results in a constitutive activation of *LIN-12* causing an egg-laying defect. Interestingly, five missense mutations in *PS1* were either less effective or ineffective in rescuing the *sel-12* mutant phenotype, indicating that these mutations alter normal presenilin function. However, the exon 10 deletion mutant of *PS1* displayed considerable rescue activity in this assay, although it was not as effective as the wild-type protein [79]. These studies suggest that the human presenilins are functionally interchangeable with *SEL-12*.

The role of SEL-12p in *LIN-12*/Notch signalling is not known. The ER-Golgi localization of the presenilins indicates that SEL-12p may act as a downstream effector activated by LIN-12 or it may function in receptor trafficking and recycling of LIN-12 [18]. In the brain, Notch1 and the presenilins were shown to colocalize in the ventricular zone during embryonic development [81]. A further clue to the neuronal role of the presenilins in the Notch pathway came from studies of transgenic mice with a targeted disruption in the *PS1* locus [82]. The *PS1* null mice suffered region-specific loss of neural progenitor cells and neurons, reminiscent of the lethal hypertrophy of the nervous system in *Notch1*$^{-/-}$ *Drosophila* [83]. Interestingly, *PS1* knockout mice also developed skeletal malformations similar to those previously observed in *Notch1*$^{-/-}$, *Dll1*$^{-/-}$ and *paraxis*$^{-/-}$ mice. Consequently, these mice died within an hour of birth, most likely due to marked deformities in the ribcage [84, 82]. Moreover, *Notch1* and *Dll1* mRNAs were found to be dramatically decreased in the developing somites of the paraxial mesoderm of the PS1 null mutant mice [84]. These data indicate that PS1p is most likely required in the Notch pathway during embryonic development and/or cellular differentiation and that its expression is necessary for the requisite temporal appearance of the *Notch1* and *Dll1* messages during somite formation.

A third potential clue to the involvement of the presenilins in the Notch pathway comes from the recent identification of PS1-interactor proteins from the catenin/Armadillo family [85]. The catenins are critical in the Wingless signalling pathway, which in turn is mutually inhibitory with the Notch pathway. The largest cytoplasmic loop (HL-6) of PS1p interacts with a newly identified catenin in neuronal cells, termed δ-catenin, while in other cell types the predominant interactor was found to be β-catenin. The exact biological function of PS1p in the Notch and Wingless pathways remains to be investigated, as well as the relationship of these pathways to the pathological effects of the *PS1* FAD mutations in AD. One possibility is that the interaction between the PS1p and δ-catenin is modulated through the Wingless signalling pathway and serves to help regulate apoptosis in neurons. It is also possible that the binding of δ-catenin or β-catenin to PS1p modulates the ability of PS1p to coordinate γ-secretase of APPp, thereby determining whether $A\beta_{40}$ or $A\beta_{42}$ is generated. In future studies it will be of great interest to determine the significance of δ-catenin binding to PS1p and to address the question of how an alteration of this interaction may contribute to AD neuropathogenesis, including the potential induction of apoptotic events and the generation of $A\beta_{42}$.

Conclusion

FAD gene defects in the genes encoding *APP* (total of six), *PS1* (total of 53) and *PS2* (total of 2) along with as yet unidentified mutations in these genes most likely account for only half of all cases of early-onset FAD. Within this group, *PS1* accounts for the greatest portion of early-onset FAD cases (roughly half of FAD patients <60 years down and most of FAD with onset <50 years old), while mutations in *APP* and *PS2* combined account for less than 1% of FAD. Data regarding the phenotypic consequences of these mutations at the molecular and biochemical levels are rapidly advancing our understanding of the aetiological and pathogenic events associated with AD neuropathogenesis. However, it is still necessary to identify the gene defects responsible for the portion of late-onset AD not accounted for by *APOE* ε4, genetic risk factors which modify the penetrance of *APOE* ε4, and the approximately 50% of early-onset FAD that is not accounted

for by *APP* and presenilin gene mutations. In a scan of the human genome using over 400 mixed early- and late-onset FAD kindreds and affected sib-pairs, we recently discovered genetic linkage to a potential FAD locus on chromosomes 3, 4, 6, 9, 12, 13 and 16 (unpublished findings). Further genetic studies are currently underway to confirm the localization of these potentially novel FAD genes. A common pathogenic event associated with all of the known FAD genes is the excessive production or accumulation of the Aβ peptide and deposition of β-amyloid in the brain. While the *APP* and presenilin gene mutations appear to operate as either 'gain-of-function' or 'dominant-negative' gene defects ultimately leading to enhanced production of Aβ or Aβ_{42}, *APOE* ε4 most likely affects the rate of aggregation or clearance of Aβ in the brain. Given that APOE normally plays a role in the storage and transport of cholesterol, a potential role for *APOE* ε4 in predisposing to AD by raising cholesterol levels has not been ruled out. The recent identification of a potential candidate for the γ-secretase that is regulated by cholesterol levels supports this hypothesis [86]. Future investigations should also help to clarify whether presenilin gene mutations lead directly to neuronal dysfunction and degeneration (with increased production of Aβ_{42} as a secondary effect) or cause neuronal cell death via increased generation of Aβ_{42} and β-amyloid deposition.

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1 Tanzi R., Gaston S., Bush A., Romano D., Pettingell W., Peppercorn J. et al. (1994) Genetic heterogenity of gene defects responsible for familial Alzheimer disease. Genetica **91:** 255–263
2 Wasco W. and Tanzi R. E. (1995) Molecular genetics of amyloid and apolipoprotein E in Alzheimer's disease. In: Neurobiology of Alzheimer's Disease, pp. 51–76, Dawbarn D. and Allen S. J. (eds), BIOS Scientific Publishers, Oxford
3 Saunders A. M., Strittmatter W. J., Schmechel D., George-Hyslop P. H., Pericak-Vance M. A., Joo S. H. et al. (1993) Association of apolipoprotein E allele 4 with late-onset familial and sporadic Alzheimer's disease. Neurology **43:** 1467–1472
4 Tanzi R. E., Vaula G., Romano D. M., Mortilla M., Huang T. L., Tupler R. G. et al. (1992) Assessment of amyloid β protein precursor gene mutations in a large set of familial and sporadic Alzheimer disease cases. Am. J. Hum. Genet. **51:** 273–282
5 Tanzi R. E., Kovacs D. M., Kim T. W., Moir R. D., Guenette S. Y. and Wasco W. (1996) The gene defects responsible for familial Alzheimer's disease. Neurobiol. Dis. **3:** 159–168
6 Rossor M. N., Fox N. C., Beck J., Campbell T. C. and Collinge J. (1996) Incomplete penetrance of familial Alzheimer's disease in a pedigree with a novel presenilin-1 gene mutation. Lancet **347:** 1560
7 Games D., Adams D., Alessandrini R., Barbour R., Berthelette P., Blackwell C. et al. (1995) Alzheimer-type neuropathology in transgenic mice overexpressing V717F β-amyloid precursor protein. Nature **373:** 523–527
8 Cai X.-D., Golde T. E. and Younkin S. G. (1993). Release of excess amyloid β protein from a mutant amyloid β protein precursor. Science **259:** 514–516
9 Suzuki N., Cheung T. T., Cai X. D., Odaka A., Otvos L. Jr., Eckman C. et al. (1994) An increased percentage of long amyloid beta protein secreted by familial amyloid beta protein precursor (beta APP717) mutants. Science **264:** 1336–1340
10 Citron M., Oltersdorf T., Haass C., McConlogue L., Hung A. Y., Seubert P. et al. (1992) Mutation of the β-amyloid precursor protein in familial Alzheimer's disease increases β-protein production. Nature **360:** 672–674
11 Hyman B. T., West H. L., Rebeck G. W., Buldyrev S. V., Mantegna R. N., Ukleja M. et al. (1995) Quantitative analysis of senile plaques in Alzheimer's disease: observation of log-normal size distribution and molecular epidemiology of differences associated with ApoE genotype and trisomy 21 (Down syndrome). Proc. Natl. Acad. Sci. USA **92:** 3586–3590
12 Bales K. R., Verina T., Dodel R. C., Du Y., Altstiel L., Bender M. et al. (1997) Lack of apolipoprotein E dramatically reduces amyloid β-peptide deposition. Nature Genet. **17:** 263–264
13 Sherrington R., Rogaev E. I., Liang Y., Rogaeva E. A., Levesque G., Ikeda M. et al. (1995) Cloning of a novel gene bearing missense mutations in early onset familial Alzheimer disease. Nature **375:** 754–760
14 Levy-Lahad E., Wasco W., Poorkaj P., Romano D. M., Oshima J., Pettingell W. H. et al. (1995) Candidate gene for the chromosome 1 familial Alzheimer's disease locus. Science **269:** 973–977
15 Rogaev E. I., Sherrington R., Rogaeva E. A., Levesque G., Ikeda M., Liang Y. et al. (1995) Familial Alzheimer's disease in kindreds with missense mutation in a gene on chomosome 1 related to the Alzheimer's disease type 3 gene. Nature **376:** 775–778
16 Hong C. S. and Koo E. H. (1997) Isolation and characterization of *Drosophila* presenilin homolog. Neuroreport **8:** 665–668
17 Boulianne G. L., Livne-Bar I., Humphreys J. M., Liang Y., Lin C., Rogaev E. et al. (1997) Cloning and characterization of the *Drosophila* presenilin homologue. Neuroreport **8:** 1025–1029
18 Levitan D. and Greenwald I. (1995) Facilitation of lin-12-mediated signalling by sel-12, a *Caenorhabditis elegans* S182 Alzheimer's disease gene. Nature **377:** 351–354
19 Xiajun L. and Greewald I. (1997) HOP-1, *Caenorhabditis elegans* presenilin, appears to be functionally redundant with SEL-12 presenilin and to facilitate LIN-12 and GLP-1 signaling. Proc. Natl. Acad. Sci. USA **94:** 12204–12209
20 Hardy J. (1997) Amyloid, the presenilins and Alzheimer's disease. Trends Neurosci. **20:** 154–159
21 Perez-Tur J., Froelich S., Prihar G., Crook R., Baker M., Duff K. et al. (1996) A mutation in Alzheimer's disease destroying a splice acceptor site in the presenilin-1 gene. Neuroreport **7:** 297–301
22 Clark R. F. and the Alzheimer's Disease Collaboration Group (1995) The structure of the presenilin 1 (S182) gene and identification of six novel mutations in early onset AD families. Nature Genet. **11:** 219–222
23 Suzuki T., Nishiyama K., Murayama S., Yamamoto A., Sato S., Kanazawa I. et al. (1996) Regional and cellular presenilin 1 gene expression in human and rat tissues. Biochem. Biophys. Res. Commun. **219:** 708–713
24 Kovacs D. M., Fausett H. J., Page K. J., Kim T.-W., Moir R. D., Merriam D. E. et al. (1996) Alzheimer associated presenilins 1 and 2: neuronal expression in brain and localization to intracellular membranes in mammalian cells. Nature Med. **2:** 224–229
25 Cribbs D. H., Chen L. S., Bende S. M. and LaFerla F. M. (1996) Widespread neuronal expression of the presenilin-1 early-onset Alzheimer's disease gene in the murine brain. Am. J. Pathol. **148:** 1797–1806

26 Lee M. K., Slunt H. H., Martin L. J., Thinakaran G., Kim G., Gandy S. E. et al. (1996) Expression of Presenilin 1 and 2 (PS1 and PS2) in human and murine tissues. J. Neurosci. **16:** 7513–7525
27 Levey A. I., Heilman C. J., Lah J. J., Nash N. R., Rees H. D., Wakai M. et al. (1997) Presenilin-1 protein expression in familial and sporadic Alzheimer's disease. Ann. Neurol. **41:** 742–753
28 Busciglio J., Hartmann H., Lorenzo A., Wong C., Baumann K., Sommer B. et al. (1997) Neuronal localization of presenilin-1 and association with amyloid plaques and neurofibrillary tangles in Alzheimer's disease. J. Neurosci. **17:** 5101–5107
29 Lah J. J., Heilman C. J., Nash N. R., Rees H. D., Yi H., Counts S. E. et al. (1997) Light and electron microscopic localization of presenilin-1 in primate brain. J. Neurosci. **17:** 1971–1980
30 Moussaoui S., Czech C., Pradier L., Blanchard V., Bonici B., Gohin M. et al. (1996) Immunohistochemical analysis of presenilin-1 expression in mouse brain. FEBS Lett. **383:** 219–222
31 Page K., Hollister R., Tanzi R. E. and Hyman B. T. (1996) In situ hybridization of presenilin 1 mRNA in Alzheimer's disease and in lesioned rat brain. Proc. Natl. Acad. Sci. USA **93:** 14020–14024
32 Walter J., Capell A., Grünberg J., Pesold B., Schindzielorz A., Prior R. et al. (1996) The Alzheimer's disease-associated presenilins are differentially phosphorylated proteins located predominantly within the endoplasmic reticulum. Mol. Med. **2:** 673–691
33 Cook D. G., Sung J. C., Golde T. E., Felsenstein K. M., Wojczyk B. S., Tanzi R. E. et al. (1996) Expression and analysis of presenilin 1 in a human neuronal system: localization in cell bodies and dendrites. Proc. Natl. Acad. Sci. USA **93:** 9223–9228
34 Strooper B., Beullens M., Contreras B., Levesque L., Craessaerts K., Cordell B. et al. (1997) Phosphorylation, subcellular localization, and membrane orientation of the Alzheimer's disease-associated presenilins. J. Biol. Chem. **272:** 3590–3598
35 Dewji N. N. and Singer S. J. (1997) Cell surface expression of the Alzheimer disease-related presenilin proteins. Proc. Natl. Acad. Sci. USA **94:** 9926–9931
36 Li J., Xu M., Zhou H., Ma J. and Potter H. (1997) Alzheimer presenilins in the nuclear membrane, interphase kinetochores and centrosomes suggest a role in chromosome segregation. Cell **90:** 917–927
37 Seeger M., Nordstedt C., Petanceska S., Kovacs D. M., Gouras G. K., Hahne S. et al. (1997) Evidence for phosphorylation and oligomeric assembly of presenilin 1. Proc. Natl. Acad. Sci. USA **94:** 5090–5094
38 Walter J., Grünberg J., Capell A., Pesold B., Schindzielorz A., Citron M. et al. (1997) Proteolytic processing of the Alzheimer disease-associated presenilin-1 gene generates an in vivo substrate for protein kinase C. Proc. Natl. Acad. Sci. USA **94:** 5349–5354
39 Doan A., Thinakaran G., Borchelt D. R., Slunt H. H., Ratovitsky T., Podlisny M. et al. (1996) Protein topology of presenilin 1. Neuron **17:** 1023–1030
40 Lehmann S., Chiesa R. and Harris D. A. (1997) Evidence for a six-transmembrane domain structure of presenilin 1. J. Biol. Chem. **272:** 12047–12051
41 Kim T.-W., Hallmark O. G., Pettingell W., Wasco W. and Tanzi R. E. (1996) Proteolytic processing of wild-type and mutant forms of presenilin 2. Neurobiol. Aging **17:** S155
42 Thinakaran G., Borchelt D., Lee M., Slunt H., Spitzer L., Kim G. et al. (1996) Endoproteolysis of presenilin 1 and accumulation of processed derivatives in vivo. Neuron **17:** 181–190
43 Mercken M., Takahashi H., Honda T., Sato K., Murayama M., Nakazato Y. et al. (1996) Characterization of human presenilin 1 using N-terminal specific monoclonal antibodies: evidence that Alzheimer mutations affect proteolytic processing. FEBS Lett. **389:** 297–303
44 Ratovitski T., Slunt H. H., Thinakaran G., Price D. L., Sisodia S. S. and Borchelt D. R. (1997) Endoproteolytic processing and stabilization of wild-type and mutant presenilin. J. Biol. Chem. **272:** 24536–24541
45 Kim T.-W., Pettingell W. H., Hallmark O. G., Moir R. D., Wasco W. and Tanzi R. E. (1997) Endoproteolytic processing and proteasomal degradation of presenilin 2 in transfected cells. J. Biol. Chem. **272:** 11006–11010(a)
46 Thinakaran G., Harris C. L., Ratovitsky T., Davenport F., Slunt H., Price D. L. et al. (1997) Evidence that levels of presenilins (PS1 and PS2) are coordinately regulated by competition for limiting cellular factors. J. Biol. Chem. **272:** 28415–28422
47 Lee M. K., Borchelt D. R., Kim G., Thinakaran G., Slunt H. H., Ratovitski T. R. et al. (1997) Hyperaccumulation of FAD-linked presenilin 1 variants in vivo. Nature Med. **3:** 723–725
48 Podlisny M. B., Citron M., Amarante P., Sherrington R., Xia W., Zhang J. et al. (1997) Presenilin proteins undergo heterogenous endoproteolysis between Thr291 and Ala299 and occur as Stable N- and C-terminal fragments in normal and Alzheimer brain tissue. Neurobiol. Dis. **3:** 325–337
49 Kim T.-W., Pettingell W. H., Jung Y.-K., Kovacs D. M. and Tanzi R. E. (1997) Alternative cleavage of Alzheimer-associated presenilins during apoptosis by caspase-3 family protease. Science **277:** 373–376
50 Loetscher H., Deuschle U., Brockhaus M., Reinhardt D., Nelboeck P., Mous J. et al. (1997) Presenilins are processed by caspase-type proteases. J. Biol. Chem. **272:** 20655–20659
51 Hartmann H., Busciglio J., Baumann K.-H., Staufenbiel M. and Yankner B. A. (1997) Developmental regulation of presenilin-1 processing in the brain suggests a role in neuronal differentiation. J. Biol. Chem. **272:** 14505–14508
52 Capell A., Saffrich R., Olivo J.-C., Meyn L., Walter J., Grünberg J. et al.. (1997) Cellular expression and proteolytic processing of presenilin proteins is developmentally regulated during neuronal differentiation. J. Neurochem. **69:** 2432–2440
53 Vito P., Ghayurt T. and D'Adamio L. (1997) Generation of anti-apoptotic presenilin-2 polypeptides by alternative transcription, proteolysis and caspase-3 cleavage. J. Biol. Chem. **272:** 28315–28320
54 Guo Q., Furukawa K., Sopher B. L., Pham D. G., Xie J., Robinson N. et al. (1996) Alzheimer's PS-1 mutation perturbs calcium homeostasis and sensitizes PC12 cells to death induced by amyloid β-peptide. Neuroreport **8:** 379–383
55 Wolozin B., Iwasaki K., Vito P., Ganjei K., Lacana E., Sunderland T. et al. (1996) PS2 participates in cellular apoptosis: constitutive activity conferred by Alzheimer mutation. Science **274:** 1710–1713
56 Deng G., Pike C. J. and Cotman C. W. (1996) Alzheimer-associated presenilin-2 confers increased sensitivity to apoptosis in PC12 cells. FEBS Lett. **397:** 50–54
57 Janicki S. and Monteiro M. J. (1997) Increased apoptosis arising from increased expression of the Alzheimer's disease-associated presenilin-2 mutation (N141I). J. Cell Biol. **139:** 485–495
58 Vito P., Lancana E. and D'Adamio L. (1996) Interfering with Apoptosis: Ca^{2+}-binding protein ALG-2 and Alzheimer's Disease Gene ALG-3. Science **271:** 521–525
59 Johnson E. M. (1994) Possible role of neuronal apoptosis in Alzheimer's disease. Neurobiol. Aging **15:** 5187–5189
60 Cotman C. W. and Anderson A. J. (1995) A potential role for apoptosis in neurodegeneration and Alzheimer's disease. Mol. Neurobiol. **10:** 19–45
61 LeBlanc A. (1996) Apoptosis and Alzheimer's disease. In: Molecular Mechanisms of Dementia, pp. 57–71, Wasco W. and Tanzi R. E. (eds), Humana Press, Totowa, NJ
62 Mellman I., Matter K., Yamamoto E., Pollack N., Roome J., Felsenstein K. et al. (1995) Mechanisms of molecular sorting in polarized cells: relevance to Alzheimer's disease. In: Alzheimer's Disease: Lessons from Cell Biology, pp. 14–26, Kosik K. S., Christen V. and Selkoe D. J. (eds), Springer, Berlin

63 Selkoe D. J. (1995) Physiological roduction and polarized secretion of the amyloid β-peptide in epithelial cells: a route to the mechanism of Alzheimer's disease. In: Alzheimer's Disease: Lessons from Cell Biology, pp. 70–77, Kosik K. S., Christen V. and Selkoe D. J. (eds), Springer, Berlin
64 Scheuner D., Eckman C., Jensen M., Song X., Citron M., Suzuki N. et al. (1996) Aβ42(43) is increased in vivo by the *PS1/2* and *APP* mutations linked to familial Alzheimer's disease. Nature Med. **2:** 864–870
65 Borchelt D. R., Thinakaran G., Eckman C. B., Lee M. K., Davenport F., Ratovitsky T. et al. (1996) Familial Alzheimer's disease-linked presenilin 1 variants elevate Aβ1-42/1-40 ratio in vitro and in vivo. Neuron **17:** 1005–1013
66 Citron M., Westaway D., Xia W., Carlson G., Diehl T., Levesque G. et al. (1996) Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid beta-protein in both transfected cells and transgenic mice. Nature Med. **3:** 67–72
67 Duff K., Eckman C., Zehr C., Yu X., Prada C.-M., Perez-Tur J. et al. (1996) Increased amyloid-β42(43) in brains of mice expressing mutant presenilin 1. Nature **383:** 710–713
68 Tomita T., Maruyama K., Saido T. C., Kume H., Shinozaki K., Tokuhiro S. et al. (1997) The presenilin 2 mutation (N141I) linked to familial Alzheimer Disease (Volga German families) increases the secretion of amyloid β protein ending at the 42nd (or 43rd) residue. Proc. Natl. Acad. Sci. USA **94:** 2025–2030
69 Xia W., Zhang J., Kholodenko D., Citron M., Podlisny M. B., Teplow D. B. et al. (1997) Enhanced production and oligomerization of the 42-residue amyloid beta protein by Chinese hamster ovary cells stably expressing mutant presenilins. J. Biol. Chem. **272:** 7977–7982(a)
70 Lemere C. A., Lopera F., Kosik K. S., Lendon C. L., Ossa J., Saido T. C. et al. (1996) The E280A presenilin 1 Alzheimer mutation produces increased Aβ42 deposition and severe cerebellar pathology. Nature Med. **2:** 1146–1150
71 Gomez-Isla T., Wasco W., Pettingell W. P., Garubhagavatula S., Schmidt D. D., Jondro P. D. et al. (1997) A novel presenilin 1 gene mutation: increased β-amyloid and neurofibrillary changes. Ann. Neurol. **41:** 809–813
72 Mann D. M. A., Iwatsubo T., Cairns N. J., Lantos P. L., Nochlin D., Sumi S. M. et al. (1996) Amyloid β protein (Aβ) deposition in chromosome 14-linked Alzheimer's disease: predominance of Aβ42(43). Ann. Neurol. **40:** 149–156
73 Hartmann T., Bieger S. C., Bruhl B., Tienari P. J., Ida N., Allsop D. et al. (1997) Distinct sites of intracellular production for Alzheimer's disease Aβ40/42 amyloid peptides. Nature Med. **3:** 1016–1020
74 Cook D. G., Forman M. S., Sung J. C., Leight S., Kolson D. L., Iwatsubo T. et al. (1997) Alzheimer's Aβ(1–42) is generated in the endoplasmic reticulum/intermediate compartment of NT2N cells. Nature Med. **3:** 1021–1023
75 Wild-Bode C., Yamazaki T., Capell A., Leimer U., Steiner H., Ihara Y. et al. (1997) Intracellular generation and accumulation of amyloid β-peptide terminating at amino acid 42. J. Biol. Chem. **272:** 16085–16088
76 Citron M., Diehl T. S., Gordon G., Biere A. L., Seubert P. and Selkoe D. J. (1996) Evidence that the 42- and 40-amino acid forms of amyloid β protein are generated from the β-amyloid precursor protein by different protease activities. Proc. Natl. Acad. Sci. USA **93:** 13170–13175
77 Weidemann A., Paliga K., Dürrwang U., Czech C., Evin G., Masters C. L. et al. (1997) Formation of stable complexes between two Alzheimer's disease gene products: presenilin-2 and β-amyloid precursor protein. Nature Med. **3:** 328–332
78 Xia W., Zhang J., Koo E. H. and Selkoe D. J. (1997) Interaction between amyloid precursor protein and presenilins in mammalian cells: implication for the pathogenesis of Alzheimer's disease. Proc. Natl. Acad. Sci. USA **94:** 8208–8213(b)
79 Levitan D., Doyle T. G., Brousseau D., Lee M. K., Thinakaran G., Slunt H. H. et al. (1996) Assessment of normal and mutant human presenilin function in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA **93:** 14940–14944
80 Baumeister R., Leimer U., Zweckbronner J., Jakubek C., Gruenberg J. and Haass C. (1997) The Sel-12 mutant phenotype of *C. elegans* is rescued independent of proteolytic processing by Wt but not mutant presenilin. Genes Funct. **1:** 149–159
81 Berezovska O., Xia M., Page K., Wasco W., Tanzi R. and Hyman B. (1997) Developmental Regulation of presenilin mRNA expression parallels Notch expression. J. Neuropathol. Exp. Neurol. **56:** 40–44
82 Shen J., Bronson R. T., Chen D. F., Xia W., Selkoe D. J. and Tonegawa S. (1997) Skeletal and CNS defects in presenilin-1-deficient mice. Cell **89:** 629–639
83 Artavanis-Tsakonas S., Matsuno K. and Fortini M. E. (1995) Notch signaling. Science **268:** 225–232
84 Wong P., Zheng H., Chen H., Becher M. W., Sirinathsinghji D. J. S., Trumbauer M. E. et al. (1997) Presenilin 1 is required for Notch1 and Dll1 expression in the paraxial mesoderm. Nature **387:** 288–292
85 Zhou J., Liyanage U., Medina M., Ho C., Simmons A. D., Lovett M. et al. (1997) Presenilin 1 interacts in brain with a novel member of the Armadillo family. Neuroreport **8:** 1489–1494
86 Rawson R. B., Zelenski N. G., Nijhawan D., Ye J., Sakai J., Hasan M. T. et al. (1997) Complementation cloning of S2P, a gene encoding a putative metalloprotease required for intramembrane cleavage of SREBPs. Mol. Cell **1:** 47–57