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Twenty Years of Presenilins—Important Proteins in Health and Disease

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Alzheimer's disease (AD) is characterized by progressive decline in cognitive functions associated with depositions of aggregated proteins in the form of extracellular plaques and neurofibrillary tangles in the brain. Extracellular plaques contain characteristic fibrils of amyloid β peptides (Aβ); tangles consist of paired helical filaments of the microtubuli-associated protein tau. Although AD manifests predominantly at ages above 65 years, rare cases show a much earlier onset of disease symptoms with very similar neuropathological characteristics. In 1995, two homologous genes were identified, in which mutations are associated with dominantly inherited familial forms of early onset AD. The genes therefore were dubbed presenilins (PS) and encode polytopic transmembrane proteins. At this time the role of these proteins in the pathogenesis of AD and their biological function in general were completely unknown. However, individuals carrying PS mutations showed alterations in the composition of different length variants of Aβ peptides in blood and cerebrospinal fluid, which indicated the potential involvement of presenilins in the metabolism of Aβ. After 20 years of intense research, the roles of presenilins in Aβ generation as well as important functions in biological processes have been identified. Presenilins represent the catalytic components of protease complexes that directly cleave the amyloid precursor protein (APP) but also many other proteins with important physiological functions. Here, the progress in presenilin research from basic characterization of their cellular functions to the targeting in clinical trials for AD therapy, and potential future directions, will be discussed.

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INTRODUCTION

Alzheimer's disease (AD) is a growing burden in aging societies and represents a major challenge in the treatment of common diseases. Currently, effective therapies that prevent progression of, or even cure, the disease are not available. Thus, the understanding of the etiology of this devastating disease is critical for future development of therapeutic or preventive strategies.

At the neuropathological level, AD is diagnosed by the combined occurrence of extracellular plaques and neurofibrillary tangles consisting of aggregated amyloid β peptides (Aβ) and hyperphosphorylated tau, respectively (1,2). Strong evidence from biochemical and molecular biological experiments as well as genetic findings indicates a critical role of Aβ aggregates in the pathogenesis of AD (3–5).

Aβ peptides have been isolated from extracellular plaques and vascular deposits from AD and Down syndrome brains and characterized by amino acid sequencing (6,7). The demonstration that the Aβ amino acid sequence is part of a much larger precursor protein already suggested that proteolytic processing would be involved in the generation of this peptide and was instrumental for subsequent work at the molecular, cell biological and *in vivo* level (8).

Aβ derives from the amyloid precursor protein (APP) by proteolytic processing involving specific proteases that were termed β- and γ-secretase (Figure 1). β-Secretase initiates Aβ generation by cleavage of APP within its ectodomain, thereby resulting in the secretion of a soluble form of APP (sAPP) and a corresponding C-terminal fragment (CTF) that is still inserted into cellular membranes (1,9). As this cleavage occurs in front of the first aspartate residue of the Aβ domain, the resultant CTF contains the full Aβ peptide sequence, which partly comprises the transmembrane domain of APP. The subsequent processing of the APP CTF by γ-secretase within the transmembrane domain eventually leads to the generation and secretion of Aβ into extracellular fluids (10,11).

IDENTIFICATION OF PRESENILIN GENES IN EARLY ONSET FAMILIAL AD

Genetic analyses of families with mendelian inheritance of early onset AD allowed the identification of causative gene mutations by positional cloning.

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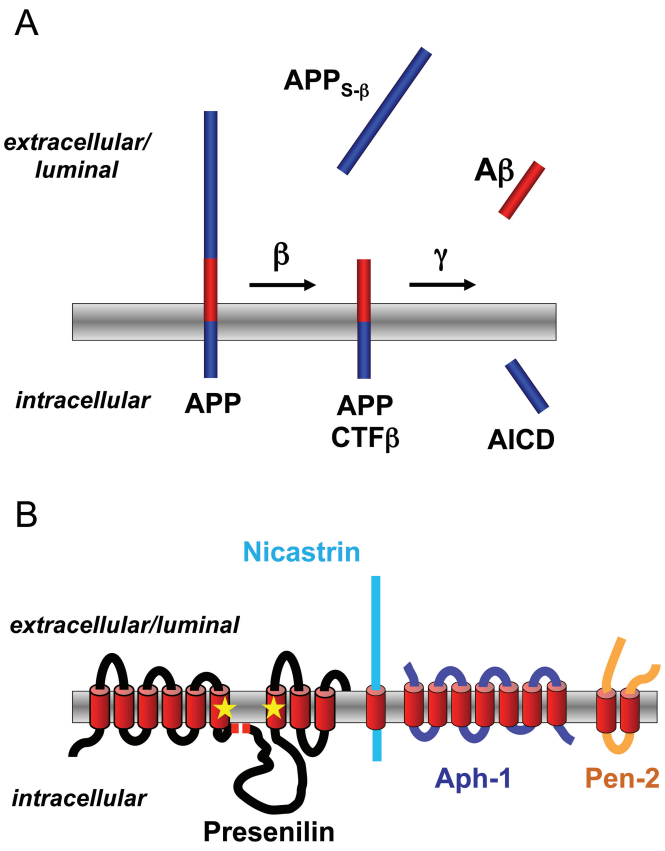

Figure 1. Schematic showing the proteolytic processing of APP and the composition of the γ-secretase complex. (A) APP is a type I transmembrane protein. β-Secretase cleaves at the N-terminus of the Aβ domain (red), resulting in the secretion of soluble APP (APPs-β) and generation of a C-terminal fragment (APP CTFβ). Subsequent cleavage of this fragment by γ-secretase liberates Aβ and the APP intracellular domain (AICD) from cellular membranes. (B) Composition of the γ-secretase complex. Presenilins represent the catalytically active proteins in the complex. Critical aspartate residues within the active site of presenilins are indicated by yellow stars. Nicastrin, Aph-1 and Pen-2 mediate assembly, substrate recognition and subcellular transport of the γ-secretase complex. See text for details.

The first gene identified was the *APP* gene itself (12), giving strong support for the amyloid hypothesis (13). In 1995, mutations in two previously uncharacterized homologous genes were identified that comprise up to 40% of all early onset familial AD (FAD) cases (14–16). Briefly, after their identification, the two genes were named presenilins (PSEN) 1 and 2. The roles of respective presenilin proteins (PS1 and PS2) in the pathogenesis of AD or their biological function were enigmatic at this time. PSEN genes are ubiquitously expressed in different tissues and show considerable conservation between mammalian and other organisms. Initial studies with plasma and primary fibroblasts of mutation carriers showed elevated levels of secreted Aβ42, while levels of Aβ40 and APP synthesis were not significantly changed compared with those from controls (17). Aβ42 has a higher aggregation propensity than Aβ40 and appears to be a critical player in triggering the deposition of amyloid plaques (3–5). Notably, plasma Aβ42 levels were also increased in presymptomatic PS mutation carriers. These results were subsequently confirmed in other cellular models as well as in transgenic mice and already suggested that presenilins could affect the metabolism of APP or Aβ itself (18–22).

INITIAL CHARACTERIZATION OF PRESENILIN PROTEINS

Early cell biological and biochemical studies aimed to characterize the subcellular localization and metabolism of PS proteins. As expected from the primary structure of PS1 and PS2, both proteins were found to be localized in different membrane compartments of cells (23–25). PS proteins were predominantly localized in the endoplasmic reticulum (ER) and the Golgi compartment. It was debated whether PS proteins could also reach additional compartments (see below). In our initial study, we also found colocalization of PS proteins with APP in the Golgi compartment (24). A binding of PS proteins with APP was later shown by coimmunoprecipitation studies (26,27).

A second part of our initial study was focused on the characterization of potential posttranslational modifications with the aim to identify potential molecular mechanisms that could regulate presenilin function. Using a heterologous expression system with transient overexpression of cDNAs encoding full-length PS1 or PS2, the proteins were detected at their expected molecular weight of about 50 kDa. Metabolic labeling with ^{32}P-orthophosphate revealed phosphate incorporation selectively into the PS2 full-length protein (24). Consistent with the presence of consensus recognition sites for casein kinases, we found that PS2 could indeed be phosphorylated by protein kinases CK1 and CK2 *in vitro* (24,28). Additional modifications, like glycosylation or sulfation, were not detected.

In contrast to the overexpressed PS proteins, very little if any endogenous protein could be detected at the expected molecular weight. A very important finding was that endogenously expressed PS1 proteins were detected as 30-kDa N-terminal fragments (NTF) and 20-kDa C-terminal fragments (CTF), which indicated a specific endoproteolytic

processing step of the full-length proteins (29–32).

We also found respective NTF and CTF of endogenously expressed PS1 in cultured cells (33). Interestingly, the PS1 CTF was found to undergo regulated phosphorylation within the hydrophilic loop region by protein kinase C, which could be stimulated by activation of muscarinic acetylcholine receptors (33,34). Since the full-length PS1 protein is not phosphorylated, these data suggested a specific regulation at the level of proteolytically processed PS1. The phosphorylation of the PS1 CTF affected its mobility in sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels, suggesting a conformational change. PS2 was found to be phosphorylated in both its NTF and CTF. In contrast to the regulated phosphorylation of the PS1 CTF by protein kinases A (PKA) and C (PKC), PS2 NTF and CTF undergo constitutive phosphorylation by protein kinases CK1 and CK2 (28). However, at this time the biological function of PS proteins and the role of phosphorylation was enigmatic (see below).

THE HUNT FOR PRESENILIN FUNCTION

Early insight into the physiologic function of PS proteins came from studies with *Caenorhabditis elegans*. Mutants of the PS homologue sel-12 in these organisms revealed an egg-laying defective phenotype closely resembling the lin-12/notch phenotype (35). Interestingly, this phenotype could be rescued by expression of the human wild-type, but not FAD mutant PS1 or PS2 (36,37). Deletion of the PS1 gene in mice led to early postnatal or late embryonic lethality associated with skeletal deformation, impaired somitogenesis and brain development (38,39). Some of these effects were similar to those in Notch mutant mice. A double knockout of both PS1 and PS2 closely resembled the Notch knockout phenotype with early embryonic lethality (40). However, PS2 knockout mice were viable and fertile and produced only mild phenotypes associated with pulmonary fibrosis and hemorrhage at higher age (40). Like APP, Notch is a type I membrane protein and has important functions in cell fate decision and differentiation during development and also during adulthood (41,42). These findings demonstrated that presenilins play fundamental physiological roles, potentially related to the function of Notch.

PS knockout mice and cells were also instrumental for understanding the role of these proteins in APP metabolism. Using neurons from PS1 knockout mice, it was demonstrated that deficiency of PS1 decreased the cleavage of APP CTFs that derive from APP by α- or β-secretory processing, and thus, the generation of Aβ (43). Interestingly, mutation of two aspartate residues localized in transmembrane domains 6 and 7 of human PS1, which are also highly conserved in PS2 and other orthologs of other species, also prevented cleavage of APP CTFs and Aβ generation. The combined evidence suggested that PS proteins themselves might exert catalytic activity and represent a novel class of aspartyl proteases that could mediate intramembraneous cleavage of APP, a characteristic feature proposed for γ-secretase (8,44). Importantly, PS proteins were also shown to mediate cleavage of Notch and thereby release the Notch intracellular domain from cellular membranes to allow its nuclear translocation and transcriptional regulation of target genes (45–47).

Previous cell biological and biochemical studies suggested that γ-secretase–dependent cleavage of APP and Notch might occur at the plasma membrane or endocytic compartments (48–52). This contrasted with the predominant localization of presenilins in the ER and early Golgi compartment, thereby raising debate about a "spatial paradox" on the differential localization of presenilins and γ-secretase activity and whether presenilin could represent γ-secretase at all (53). However, other studies demonstrated the presence of presenilins (and γ-secretase) in different secretory and endocytic compartments as well as at the plasma membrane (54–56).

PRESENILINS AS THE CORE COMPONENT OF THE γ-SECRETASE COMPLEX

As mentioned above, presenilins are mainly found as stable N- and C-terminal fragments, whereas the full-length proteins appear to be instable (29,31,32,57,58). Several early studies suggested that PS NTF and CTF exist in larger complexes, probably together with additional components (59–61). Protein interaction studies then revealed that PS proteins could associate with members of the catenin family together with glycogen synthase kinase 3β (GSK3β) and tau (62–65). Whether this interaction affects the activity of γ-secretase remains unclear (see below).

The first protein identified to be critically involved in γ-secretase activity of presenilin was a type I protein called Nicastrin (66). Functional screens for Notch signaling in *C. elegans* led to the identification of two additional proteins called anterior pharynx defective 1 (aph-1) and presenlin-enhancer-2 (pen-2) (67). Later studies showed that these three proteins together with presenilins are required and sufficient to constitute the active γ-secretase complex (68,69) (Figure 1). While presenilins represent the catalytically active component of the γ-secretase complex, the other proteins mediate complex assembly, subcellular trafficking and substrate recognition (70–73).

Besides APP and Notch, more than 90 substrate proteins for γ-secretase have been identified (74). The vast majority of them, like APP and Notch, are type I membrane proteins. However, a physiological role for the cleavage of most proteins remains to be determined. Given the high number of substrates, γ-secretase might also play a more general role in the degradation of type I membrane proteins (74,75). Cleavage of the transmembrane domain could liberate extracellular peptides and intracellular domains from cellular membranes for further degradation.

EFFECT OF FAD-ASSOCIATED MUTATIONS

Since the identification of mutations in the presenilins as a major cause of famil-

ial early onset AD, the effect of such mutations on the generation of Aβ and activity of γ-secretase has been of prime interest. Today, more than 200 different mutations in PS1 and PS2 have been identified (www.alzforum.org/mutations). As described above, the common effect of FAD-associated mutations is an increased ratio of Aβ42/Aβ40 peptides. However, it is still unclear whether all of the pathogenic mutations act similarly. Although some of the mutations studied so far might increase the production of Aβ42, most mutations decreased the secretion of Aβ40 peptides (76,77). PS FAD–associated mutations also decrease the secretion of Aβ40 more strongly than Aβ42 in induced pluripotent or embryonic stem cell–derived human neurons, further supporting a partial loss of γ-secretase function of PS FAD mutants (78,79).

Whether the mutations cause a gain of function (increased production of Aβ42) or a loss of function (decreased production of Aβ40) is of great importance considering the development of drugs that modulate γ-secretase activity for treatment or prevention of AD (see below). γ-Secretase cleaves the APP CTF initially at the interface of the transmembrane domain and the cytoplasmic domain, thereby liberating the APP intracellular domain (AICD) fragment into the cytosol. This so-called ε-cleavage, however, can occur at least at two different positions leading to the generation of "long" Aβ peptides (Aβ48 or Aβ49). Elegant and comprehensive analyses on the subsequent processing steps indicate that γ-secretase processes these long Aβ peptides in steps of three amino acids, leading to two product lines that could end at Aβ42 and Aβ40, respectively (48→45→42 or 49→46→43→40) (80). It is interesting that certain transition-state analogs used as γ-secretase inhibitors led to accumulation of longer Aβ species *in vitro*, suggesting they affect the "processivity" of γ-secretase. A similar effect has also been observed for certain γ-secretase mutations (80).

PRESENILINS MIGHT EXERT γ-SECRETASE–INDEPENDENT FUNCTION

Since their identification, presenilins have also been related to biological functions that might be independent from their activity in γ-secretase, including the regulation of apoptosis, cellular calcium homeostasis, protein transport and signaling (81,82).

Apoptosis

A screen for genes that inhibit T-cell receptor–induced apoptosis revealed a cDNA fragment encoding the C-terminal amino acids 346–449 of PS2, suggesting that PS2 might exert an anti-apoptotic function (83). However, overexpression of PS2 full-length rather promoted apoptosis in neuronally differentiated PC12 cells (62). While the exact role of PS2 in the regulation of apoptosis was enigmatic, additional support for a connection of PS proteins to apoptosis came from the observation that both PS1 and PS2 are cleaved by caspases-3 and -7, two important proteases in the execution phase of programmed cell death (84–87).

Building on the initial observations on the phosphorylation of PS2 (24), we assessed a potential role in the regulation of apoptosis. Phosphorylation sites within the PS2 CTF were identified at Ser327 and Ser330 (88). Interestingly, both sites are localized directly at the cleavage sites for caspases. The phosphorylation of PS2 CTF at these sites strongly decreased the caspase-mediated processing and also retarded the progression of apoptosis (88). A very similar effect was also identified at the phosphorylation of the PS1 CTF at Ser346 (89).

In addition to the phosphorylation of Ser346 by PKA or PKC, the PS1 CTF can also be phosphorylated at Ser353 and Ser357 by GSK3β (90,91). Phosphorylation at these sites strongly decreased the interaction of PS1 with β-catenin (92). This finding was consistent with the initial identification of β-catenin as an interaction partner of PS1 (64,93). The function of PS1 in β-catenin metabolism and signaling appears to be independent of its catalytic activity (90,94–96). Additional phosphorylation sites have also been identified. However, mutations of the respective sites did not affect APP processing (97). Whether the phosphorylation of PS proteins could affect the subcellular transport, activity of γ-secretase or additional functions remains to be determined.

Calcium Homeostasis

The polytopic transmembrane structure with 9 predicted transmembrane domains led to the speculation that PS proteins could exert channel activity. Interestingly, studies with heterologous expression systems and neurons from transgenic mice as well as with FAD patient–derived fibroblasts revealed a role of PS proteins in Ca^{2+} signaling. Cells endogenously expressing FAD mutant PS1 or PS2 showed increased release of Ca^{2+} from ER stores and also altered activity and expression of inositol trisphosphate (IP3) and ryanodine receptors (98–102). However, the molecular mechanisms underlying these observations are still under debate.

PS proteins could interact with these receptors and regulate their gating properties and/or subcellular localization. In addition, PS-dependent effects on the expression and turnover of these Ca^{2+} channels have also been described (100–102). Because PS FAD mutants also affect the proteolytic processing of APP and the ratio of Aβ42/40, cytosolic Ca^{2+} levels could also be affected by increased membrane perturbation by aggregated forms of Aβ. In addition, the APP intracellular domain generated by γ-secretase activity could also alter the expression of IP3 and ryanidone receptors by transcriptional regulation (103,104). The Notch ICD has also been shown to regulate Ca^{2+} signaling in hippocampal neurons and thereby affect synaptic plasticity, learning and memory, and Ca^{2+}-dependent cell death (55).

The PS proteins themselves might also function as Ca^{2+} channels. Tu *et al*. have shown that PS proteins represent low conduction ER leak channels (105). Inter-

estingly, this function is exerted selectively by full-length PS without or prior to assembly into the γ-secretase complex (105). However, other studies did not show altered Ca^{2+} leak from the ER by PS mutations (100,106–109).

Although the exact mechanisms that contribute to PS-dependent Ca^{2+} signaling remain to be determined, aberrant Ca^{2+} mobilization from ER or other cellular stores might affect synaptic plasticity, and thus learning and memory. Elevated cytosolic Ca^{2+} concentrations could sensitize neurons to Aβ-mediated toxicity and other stressors and promote neuronal degeneration and cell death (100–112).

PROTEIN TRANSPORT AND METABOLISM

Several studies also indicate that PS could affect subcellular transport and degradation of select proteins. In PS knockout cells, the transport of TrkB was found to be reduced (113). In addition, the targeting of telencephalin and v_0ATPase to endosomal and lysosomal compartments is impaired in PS-deficient cells (114,115). Interestingly, some indications suggest that these effects are independent of the catalytic activity of γ-secretase. Whether and how these mechanisms contribute to the impairment of lysosomal function, and thus also autophagy, is debated and requires further investigation (116–118). In general, the differentiation of γ-secretase activity dependent and independent PS function is challenging (82), and might require knockin models with inactive PS proteins at the endogenous expression level.

TRANSLATIONAL ASPECTS

Given the fundamental role of γ-secretase in the production of Aβ, this protein complex is considered as an interesting target in AD therapy or prevention. Different inhibitors have been developed that efficiently decrease Aβ generation *in vitro* and in mouse models of AD. Consequently, several clinical (phase II and III) trials have been performed and are ongoing (http://www.alzforum.org/therapeutics) (119). Unfortunately, so far no trial has shown beneficial effects on cognitive performance. Rather, detrimental outcomes were observed (120). This might at least in part be due to the broad substrate spectrum of γ-secretase and its important physiological functions that would also be affected by strong inhibition of its enzymatic activity (121,122). It needs to be kept in mind that presenilins are essential not only for embryonic development, but also in adulthood. The conditional deletion of PS proteins in neurons prevents Aβ generation but leads to age-dependent neurodegeneration in mouse brain and early death (123).

It is important to note that these findings and the sobering outcome of the clinical trials does not mean that γ-secretase is not a potential target for future AD therapy. Careful consideration of dosing and the development of more selective compounds that could target individual γ-secretase complexes or activities will help in future approaches. Indeed, the identification of compounds that could modulate γ-secretase specificity or processivity rather than generally inhibit its enzymatic activity holds great promise for further drug development targeting γ-secretase. Here, the observation that certain nonsteroidal anti-inflammatory drugs (NSAIDs) could selectively decrease the production of Aβ42, without affecting Aβ40, was instrumental for the development of γ-secretase modulators (GSMs) (124). However, as with the γ-secretase inhibitors, GSMs have not proven beneficial in the treatment of AD so far (125).

FUTURE CONSIDERATIONS

Despite the strong progress that has been made in presenilin and γ-secretase research in the past 20 years, we can only surmise the complex biological and pathophysiological roles of this enzyme. Further research will help to elucidate the functional relevance of the γ-secretase–mediated cleavages of individual protein substrates in different cell types and organs. We still know very little about the transcriptional, posttranscriptional and posttranslational mechanisms that regulate expression, trafficking and activity of presenilins and their complex partners. The existence of two major isoforms of PS (PS1 and PS2) and Aph-1 (Aph-1a and Aph-1b), several additional splice variants, and posttranslational modifications allows the formation of several distinct γ-secretase complexes that might have distinct, albeit partially redundant biological activities.

Notably, γ-secretase also regulates the metabolism of other important risk factors of AD, like apolipoprotein E (126) and the triggering receptor expressed on myeloid cells 2 (Trem2) (127,128). Thus, it will be interesting to assess whether γ-secretase contributes to the pathogenesis of AD not only via the generation of Aβ, but also by affecting additional pathways involved in the pathogenesis of the most common late onset form of AD.

The recent progress in elucidation of the molecular structure of the γ-secretase complex (129–131) will also allow rational drug designing to improve the characteristics of compounds to modulate γ-secretase function for future targeting of this enzyme in AD therapy or prevention.

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DISCLOSURE

The author declares he has no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

REFERENCES

1. Selkoe D, Mandelkow E, Holtzman D. (2012) Deciphering Alzheimer disease. *Cold Spring Harb. Perspect. Med*. 2:a011460.
2. Querfurth HW, LaFerla FM. (2010) Alzheimer's disease. *N. Engl. J. Med*. 362:329–44.
3. Kennedy JL, Farrer LA, Andreasen NC, Mayeux R, St George-Hyslop P. (2003) The genetics of adult-onset neuropsychiatric disease: complexities and conundra? *Science*. 302:822–6.
4. Tanzi RE. (2012) The genetics of Alzheimer disease. *Cold Spring Harb. Perspect. Med*. 2:a006296.
5. Goate A, Hardy J. (2012) Twenty years of Alzheimer's disease-causing mutations. *J. Neurochem*. 120(Suppl 1):3–8.
6. Glenner GG, Wong CW. (1984) Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem. Biophys. Res. Commun*. 120:885–90.
7. Masters CL, *et al*. (1985) Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc. Natl. Acad. Sci. U. S. A*. 82:4245–9.
8. Kang J, *et al*. (1987) The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature*. 325:733–6.
9. Haass C, Kaether C, Thinakaran G, Sisodia S. (2012) Trafficking and proteolytic processing of APP. *Cold Spring Harb. Perspect. Med*. 2:a006270.
10. De Strooper B, Vassar R, Golde T. (2010) The secretases: enzymes with therapeutic potential in Alzheimer disease. *Nat. Rev. Neurol*. 6:99–107.
11. Walter J, Kaether C, Steiner H, Haass C. (2001) The cell biology of Alzheimer's disease: uncovering the secrets of secretases. *Curr. Opin. Neurobiol*. 11:585–90.
12. Goate et al. (1991) Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature*. 349:704–6.
13. Hardy J, Selkoe DJ. (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science*. 297:353–6.
14. Sherrington R, *et al*. (1995) Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature*. 375:754–60.
15. Rogaev EI, *et al*. (1995) Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. *Nature*. 376:775–8.
16. Levy-Lahad E, *et al*. (1995) Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science*. 269:973–7.
17. Scheuner D, *et al*. (1996) Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat. Med*. 2:864–70.
18. Borchelt DR, *et al*. (1996) Familial Alzheimer's disease-linked presenilin 1 variants elevate Abeta1–42/1–40 ratio in vitro and in vivo. *Neuron*. 17:1005–13.
19. Holcomb L, *et al*. (1998) Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nat. Med*. 4:97–100.
20. Tomita T, *et al*. (1997) The presenilin 2 mutation (N141I) linked to familial Alzheimer disease (Volga German families) increases the secretion of amyloid beta protein ending at the 42nd (or 43rd) residue. *Proc. Natl. Acad. Sci. U. S. A*. 94:2025–30.
21. Oyama F, *et al*. (1998) Mutant presenilin 2 transgenic mouse: effect on an age-dependent increase of amyloid beta-protein 42 in the brain. *J. Neurochem*. 71:313–22.
22. Mehta ND, *et al*. (1998) Increased Abeta42(43) from cell lines expressing presenilin 1 mutations. *Ann. Neurol*. 43:256–8.
23. Kovacs DM, *et al*. (1996) Alzheimer-associated presenilins 1 and 2: neuronal expression in brain and localization to intracellular membranes in mammalian cells. *Nat. Med*. 2:224–9.
24. Walter J, *et al*. (1996) The Alzheimer's disease-associated presenilins are differentially phosphorylated proteins located predominantly within the endoplasmic reticulum. *Mol. Med*. 2:673–91.
25. De Strooper B, *et al*. (1997) Phosphorylation, subcellular localization, and membrane orientation of the Alzheimer's disease-associated presenilins. *J. Biol. Chem*. 272:3590–8.
26. Weidemann A, *et al*. (1997) Formation of stable complexes between two Alzheimer's disease gene products: presenilin-2 and beta-amyloid precursor protein. *Nat. Med*. 3:328–32.
27. Xia W, Zhang J, Perez R, Koo EH, Selkoe DJ. (1997) Interaction between amyloid precursor protein and presenilins in mammalian cells: implications for the pathogenesis of Alzheimer disease. *Proc. Natl. Acad. Sci. U. S. A*. 94:8208–13.
28. Walter J, Grunberg J, Schindzielorz A, Haass C. (1998) Proteolytic fragments of the Alzheimer's disease associated presenilins-1 and -2 are phosphorylated in vivo by distinct cellular mechanisms. *Biochemistry*. 37:5961–7.
29. Thinakaran G, *et al*. (1996) Endoproteolysis of presenilin 1 and accumulation of processed derivatives in vivo. *Neuron*. 17:181–90.
30. Lee MK, *et al*. (1996) Expression of presenilin 1 and 2 (PS1 and PS2) in human and murine tissues. *J. Neurosci*. 16:7513–25.
31. Mercken M, *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.
32. Ward RV, *et al*. (1996) Presenilin-1 is processed into two major cleavage products in neuronal cell lines. *Neurodegeneration*. 5:293–8.
33. Walter J, *et al*. (1997) Proteolytic processing of the Alzheimer disease-associated presenilin-1 generates an in vivo substrate for protein kinase C. *Proc. Natl. Acad. Sci. U. S. A*. 94:5349–54.
34. Seeger M, *et al*. (1997) Evidence for phosphorylation and oligomeric assembly of presenilin 1. *Proc. Natl. Acad. Sci. U. S. A*. 94:5090–4.
35. Levitan D, Greenwald I. (1995) Facilitation of lin-12-mediated signalling by sel-12, a Caenorhabditis elegans S182 Alzheimer's disease gene. *Nature*. 377:351–4.
36. Levitan D, *et al*. (1996) Assessment of normal and mutant human presenilin function in Caenorhabditis elegans. *Proc. Natl. Acad. Sci. U. S. A*. 93:14940–4.
37. Baumeister R, *et al*. (1997) Human presenilin-1, but not familial Alzheimer's disease (FAD) mutants, facilitate Caenorhabditis elegans Notch signalling independently of proteolytic processing. *Genes Funct*. 1:149–59.
38. Shen J, *et al*. (1997) Skeletal and CNS defects in Presenilin-1-deficient mice. *Cell*. 89:629–39.
39. Wong PC, *et al*. (1997) Presenilin 1 is required for Notch1 and DII1 expression in the paraxial mesoderm. *Nature*. 387:288–92.
40. Herreman A, *et al*.(1999) Presenilin 2 deficiency causes a mild pulmonary phenotype and no changes in amyloid precursor protein processing but enhances the embryonic lethal phenotype of presenilin 1 deficiency. *Proc. Natl. Acad. Sci. U. S. A*. 96:11872–7.
41. Mumm JS, Kopan R(2000) Notch signaling: from the outside in. *Dev. Biol*. 228:151–65.
42. Hori K, Sen A, Artavanis-Tsakonas S. (2013) Notch signaling at a glance. *J. Cell Sci*. 126:2135–40.
43. De Strooper B, *et al*. (1998) Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature*. 391:387–90.
44. Wolfe MS, et al. (1999) Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity. *Nature*. 398:513–7.
45. Struhl G, Greenwald I. (1999) Presenilin is required for activity and nuclear access of Notch in Drosophila. *Nature*. 398:522–5.
46. De Strooper B, *et al*. (1999) A presenilin-1-dependent gamma-secretase-like protease mediates release of Notch intracellular domain. *Nature*. 398:518–22.
47. Ye Y, Lukinova N, Fortini ME. (1999) Neurogenic phenotypes and altered Notch processing in Drosophila Presenilin mutants. *Nature*. 398:525–9.
48. Struhl G, Adachi A. (1998) Nuclear access and action of notch in vivo. *Cell*. 93:649–60.
49. Koo EH, Squazzo SL. (1994) Evidence that production and release of amyloid beta-protein involves the endocytic pathway. *J. Biol. Chem*. 269:17386–9.
50. Lai A, Sisodia SS, Trowbridge IS. (1995) Characterization of sorting signals in the beta-amyloid precursor protein cytoplasmic domain. *J. Biol. Chem*. 270:3565–73.
51. Yamazaki T, Selkoe DJ, Koo EH. (1995) Trafficking of cell surface beta-amyloid precursor protein: retrograde and transcytotic transport in cultured neurons. *J. Cell Biol*. 129:431–42.
52. Wild-Bode C, *et al*. (1997) Intracellular generation and accumulation of amyloid beta-peptide terminating at amino acid 42. *J. Biol. Chem*. 272:16085–8.
53. Annaert WG, *et al*. (1999) Presenilin 1 controls gamma-secretase processing of amyloid precursor protein in pre-Golgi compartments of hippocampal neurons. *J. Cell Biol*. 147:277–94.
54. Kaether C, *et al*. (2002) Presenilin-1 affects traf-

ficking and processing of betaAPP and is targeted in a complex with nicastrin to the plasma membrane. *J. Cell Biol*. 158:551–61.
55. Wang Y, *et al*. (2004) Involvement of Notch signaling in hippocampal synaptic plasticity. *Proc. Natl. Acad. Sci. U. S. A*. 101:9458–62.
56. Vetrivel KS, *et al*. (2004) Association of gamma-secretase with lipid rafts in post-Golgi and endosome membranes. *J. Biol. Chem*. 279:44945–54.
57. Podlisny MB, *et al*. (1997) Presenilin proteins undergo heterogeneous endoproteolysis between Thr291 and Ala299 and occur as stable N- and C-terminal fragments in normal and Alzheimer brain tissue. *Neurobiol. Dis*. 3:325–37.
58. Marambaud P, Ancolio K, Lopez-Perez E, Checler F. (1998) Proteasome inhibitors prevent the degradation of familial Alzheimer's disease-linked presenilin 1 and potentiate A beta 42 recovery from human cells. *Mol. Med*. 4:147–57.
59. Capell A, *et al*. (1998) The proteolytic fragments of the Alzheimer's disease-associated presenilin-1 form heterodimers and occur as a 100–150-kDa molecular mass complex. *J. Biol. Chem*. 273:3205–11.
60. Steiner H, *et al*. (1998) Expression of Alzheimer's disease-associated presenilin-1 is controlled by proteolytic degradation and complex formation. *J. Biol. Chem*. 273:32322–31.
61. Thinakaran G, *et al*. (1997) Evidence that levels of presenilins (PS1 and PS2) are coordinately regulated by competition for limiting cellular factors. *J. Biol. Chem*. 272:28415–22.
62. Wolozin B, et al. (1996) Participation of presenilin 2 in apoptosis: enhanced basal activity conferred by an Alzheimer mutation. *Science*. 274:1710–3.
63. Zhou J, et al. (1997) Presenilin 1 interaction in the brain with a novel member of the Armadillo family. *Neuroreport*. 8:1489–94.
64. Yu G, *et al*. (1998) The presenilin 1 protein is a component of a high molecular weight intracellular complex that contains beta-catenin. *J. Biol. Chem*. 273:16470–5.
65. Takashima A, *et al*. (1998) Presenilin 1 associates with glycogen synthase kinase-3beta and its substrate tau. *Proc. Natl. Acad. Sci. U. S. A*. 95:9637–41.
66. Yu G, *et al*. (2000) Nicastrin modulates presenilin-mediated notch/glp-1 signal transduction and betaAPP processing. *Nature*. 407:48–54.
67. Francis R, *et al*. (2002) aph-1 and pen-2 are required for Notch pathway signaling, gamma-secretase cleavage of betaAPP, and presenilin protein accumulation. *Dev. Cell*. 3:85–97.
68. Edbauer D, *et al*. (2003) Reconstitution of gamma-secretase activity. *Nat. Cell Biol*. 5:486–8.
69. Kimberly WT, *et al*. (2003) Gamma-secretase is a membrane protein complex comprised of presenilin, nicastrin, Aph-1, and Pen-2. *Proc. Natl. Acad. Sci. U. S. A*. 100:6382–7.
70. Takasugi N, *et al*. (2003) The role of presenilin cofactors in the gamma-secretase complex. *Nature*. 422:438–41.
71. Haass C, Steiner H. (2002) Alzheimer disease gamma-secretase: a complex story of GxGD-type presenilin proteases. *Trends Cell. Biol*. 12:556–62.
72. De Strooper B, Annaert W. (2010) Novel research horizons for presenilins and gamma-secretases in cell biology and disease. *Annu. Rev. Cell Dev. Biol*. 26:235–60.
73. St George-Hyslop P, Fraser PE. (2012) Assembly of the presenilin gamma-/epsilon-secretase complex. *J. Neurochem*. 120(Suppl 1):84–8.
74. Haapasalo A, Kovacs DM. (2011) The many substrates of presenilin/gamma-secretase. *J. Alzheimers Dis*. 25:3–28.
75. Kopan R, Ilagan MX. (2004) Gamma-secretase: proteasome of the membrane? *Nat. Rev. Mol. Cell. Biol*. 5:499–504.
76. Wolfe MS. (2007) When loss is gain: reduced presenilin proteolytic function leads to increased Abeta42/Abeta40. Talking Point on the role of presenilin mutations in Alzheimer disease. *EMBO Rep*. 8:136–40.
77. Shen J, Kelleher RJ III. (2007) The presenilin hypothesis of Alzheimer's disease: evidence for a loss-of-function pathogenic mechanism. *Proc. Natl. Acad. Sci. U. S. A*. 104:403–9.
78. Mertens J, *et al*. (2013) APP processing in human pluripotent stem cell-derived neurons is resistant to NSAID-based gamma-secretase modulation. *Stem Cell Reports*. 1:491–8.
79. Koch P, *et al*. (2012) Presenilin-1 L166P mutant human pluripotent stem cell-derived neurons exhibit partial loss of gamma-secretase activity in endogenous amyloid-beta generation. *Am. J. Pathol*. 180:2404–16.
80. Morishima-Kawashima M. (2014) Molecular mechanism of the intramembrane cleavage of the beta-carboxyl terminal fragment of amyloid precursor protein by gamma-secretase. *Front. Physiol*. 5:463.
81. Pimplikar SW, Nixon RA, Robakis NK, Shen J, Tsai LH. (2010) Amyloid-independent mechanisms in Alzheimer's disease pathogenesis. *J. Neurosci*. 30:14946–54.
82. Robakis NK. (2011) Mechanisms of AD neurodegeneration may be independent of Abeta and its derivatives. *Neurobiol. Aging*. 32:372–9.
83. Vito P, Lacana E, D'Adamio L. (1996) Interfering with apoptosis: Ca(2+)-binding protein ALG-2 and Alzheimer's disease gene ALG-3. *Science*. 271:521–5.
84. Grunberg J, *et al*. (1998) Alzheimer's disease associated presenilin-1 holoprotein and its 18–20 kDa C-terminal fragment are death substrates for proteases of the caspase family. *Biochemistry*. 37:2263–70.
85. Kim TW, Pettingell WH, Jung YK, Kovacs DM, Tanzi RE. (1997) Alternative cleavage of Alzheimer-associated presenilins during apoptosis by a caspase-3 family protease. *Science*. 277:373–6.
86. Loetscher H, *et al*. (1997) Presenilins are processed by caspase-type proteases. *J. Biol. Chem*. 272:20655–9.
87. Vito P, Ghayur T, D'Adamio L. (1997) Generation of anti-apoptotic presenilin-2 polypeptides by alternative transcription, proteolysis, and caspase-3 cleavage. *J. Biol. Chem*. 272:28315–20.
88. Walter J, Schindzielorz A, Grunberg J, Haass C. (1999) Phosphorylation of presenilin-2 regulates its cleavage by caspases and retards progression of apoptosis. *Proc. Natl. Acad. Sci. U. S. A*. 96:1391–6.
89. Fluhrer R, *et al*. (2003) Identification of a beta-secretase activity, which truncates amyloid beta-peptide after its presenilin-dependent generation. *J. Biol. Chem*. 278:5531–8.
90. Kirschenbaum F, Hsu SC, Cordell B, McCarthy JV. (2001) Glycogen synthase kinase-3beta regulates presenilin 1 C-terminal fragment levels. *J. Biol. Chem*. 276:30701–7.
91. Kirschenbaum F, Hsu SC, Cordell B, McCarthy JV. (2001) Substitution of a glycogen synthase kinase-3beta phosphorylation site in presenilin 1 separates presenilin function from beta-catenin signaling. *J. Biol. Chem*. 276:7366–75.
92. Prager K, *et al*. (2007) A structural switch of presenilin 1 by glycogen synthase kinase 3beta-mediated phosphorylation regulates the interaction with beta-catenin and its nuclear signaling. *J. Biol. Chem*. 282:14083–93.
93. Zhang Z, *et al*. (1998) Destabilization of beta-catenin by mutations in presenilin-1 potentiates neuronal apoptosis. *Nature*. 395:698–702.
94. Nishimura M, *et al*. (1999) Presenilin mutations associated with Alzheimer disease cause defective intracellular trafficking of beta-catenin, a component of the presenilin protein complex. *Nat. Med*. 5:164–9.
95. Kang DE, *et al*. (1999) Presenilin 1 facilitates the constitutive turnover of beta-catenin: differential activity of Alzheimer's disease-linked PS1 mutants in the beta-catenin-signaling pathway. *J. Neurosci*. 19:4229–37.
96. Kang DE, *et al*. (2002) Presenilin couples the paired phosphorylation of beta-catenin independent of axin: implications for beta-catenin activation in tumorigenesis. *Cell*. 110:751–62.
97. Matz A, *et al*. (2015) Identification of new Presenilin-1 phosphosites: implication for gamma-secretase activity and Abeta production. *J. Neurochem*. 133:409–21.
98. Guo Q, Robinson N, Mattson MP. (1998) Secreted beta-amyloid precursor protein counteracts the proapoptotic action of mutant presenilin-1 by activation of NF-kappaB and stabilization of calcium homeostasis. *J. Biol. Chem*. 273:12341–51.
99. Leissring MA, *et al*. (2000) Capacitative calcium entry deficits and elevated luminal calcium content in mutant presenilin-1 knockin mice. *J. Cell Biol*. 149:793–8.
100. Honarnejad K, Herms J. (2012) Presenilins: role in calcium homeostasis. *Int. J. Biochem. Cell Biol*. 44:1983–6.
101. LaFerla FM. (2002) Calcium dyshomeostasis and intracellular signalling in Alzheimer's disease. *Nat. Rev. Neurosci*. 3:862–72.
102. Mattson MP. (2010) ER calcium and Alzheimer's disease: in a state of flux. *Sci. Signal*. 3:e10.
103. Leissring MA, *et al*. (2002) A physiologic signaling role for the gamma -secretase-derived intra-

cellular fragment of APP. *Proc. Natl. Acad. Sci. U. S. A.* 99:4697–702.
104. Chan SL, Mayne M, Holden CP, Geiger JD, Mattson MP. (2000) Presenilin-1 mutations increase levels of ryanodine receptors and calcium release in PC12 cells and cortical neurons. *J. Biol. Chem.* 275:18195–200.
105. Tu H, *et al.* (2006) Presenilins form ER Ca2+ leak channels, a function disrupted by familial Alzheimer's disease-linked mutations. *Cell.* 126:981–93.
106. Shilling D, Mak DO, Kang DE, Foskett JK. (2012) Lack of evidence for presenilins as endoplasmic reticulum Ca2+ leak channels. *J. Biol. Chem.* 287:10933–44.
107. Brunello L, *et al.* (2009) Presenilin-2 dampens intracellular Ca2+ stores by increasing Ca2+ leakage and reducing Ca2+ uptake. *J. Cell. Mol. Med.* 13:3358–69.
108. Cheung KH, *et al.* (2008) Mechanism of Ca2+ disruption in Alzheimer's disease by presenilin regulation of InsP3 receptor channel gating. *Neuron.* 58:871–83.
109. McCombs JE, Gibson EA, Palmer AE. (2010) Using a genetically targeted sensor to investigate the role of presenilin-1 in ER Ca2+ levels and dynamics. *Mol. Biosyst.* 6:1640–9.
110. Popugaeva E, Bezprozvanny I. (2013) Role of endoplasmic reticulum Ca2+ signaling in the pathogenesis of Alzheimer disease. *Front. Mol. Neurosci.* 6:29.
111. Bezprozvanny I, Mattson MP. (2008) Neuronal calcium mishandling and the pathogenesis of Alzheimer's disease. *Trends Neurosci.* 31:454–63.
112. Green KN, LaFerla FM. (2008) Linking calcium to Abeta and Alzheimer's disease. *Neuron.* 59:190–4.
113. Naruse S, *et al.* (1998) Effects of PS1 deficiency on membrane protein trafficking in neurons. *Neuron.* 21:1213–21.
114. Esselens C, *et al.* (2004) Presenilin 1 mediates the turnover of telencephalin in hippocampal neurons via an autophagic degradative pathway. *J. Cell Biol.* 166:1041–54.
115. Lee JH, *et al.* (2010) Lysosomal proteolysis and autophagy require presenilin 1 and are disrupted by Alzheimer-related PS1 mutations. *Cell.* 141:1146–58.
116. Coen K, *et al.* (2012) Lysosomal calcium homeostasis defects, not proton pump defects, cause endo-lysosomal dysfunction in PSEN-deficient cells. *J. Cell Biol.* 198:23–35.
117. Zhang X, *et al.* (2012) A role for presenilins in autophagy revisited: normal acidification of lysosomes in cells lacking PSEN1 and PSEN2. *J. Neurosci.* 32:8633–48.
118. Orr ME, Oddo S. (2013) Autophagic/lysosomal dysfunction in Alzheimer's disease. *Alzheimers Res. Ther.* 5:53.
119. Selkoe DJ. (2013) The therapeutics of Alzheimer's disease: where we stand and where we are heading. *Ann. Neurol.* 74:328–36.
120. Doody RS, *et al.* (2013) A phase 3 trial of semagacestat for treatment of Alzheimer's disease. *N. Engl. J. Med.* 369:341–50.
121. De Strooper B. (2014) Lessons from a failed gamma-secretase Alzheimer trial. *Cell.* 159:721–6.
122. Karran E, Hardy J. (2014) A critique of the drug discovery and phase 3 clinical programs targeting the amyloid hypothesis for Alzheimer disease. *Ann. Neurol.* 76:185–205.
123. Saura CA, *et al.* (2004) Loss of presenilin function causes impairments of memory and synaptic plasticity followed by age-dependent neurodegeneration. *Neuron.* 42:23–36.
124. Weggen S, *et al.* (2001) A subset of NSAIDs lower amyloidogenic Abeta42 independently of cyclooxygenase activity. *Nature.* 414:212–6.
125. Mullane K, Williams M. (2013) Alzheimer's therapeutics: continued clinical failures question the validity of the amyloid hypothesis-but what lies beyond? *Biochem. Pharmacol.* 85:289–305.
126. Tamboli IY, *et al.* (2008) Loss of gamma-secretase function impairs endocytosis of lipoprotein particles and membrane cholesterol homeostasis. *J. Neurosci.* 28:12097–106.
127. Wunderlich P, *et al.* (2013) Sequential proteolytic processing of the triggering receptor expressed on myeloid cells-2 (TREM2) protein by ectodomain shedding and gamma-secretase-dependent intramembranous cleavage. *J. Biol. Chem.* 288:33027–36.
128. Kleinberger G, *et al.* (2014) TREM2 mutations implicated in neurodegeneration impair cell surface transport and phagocytosis. *Sci. Transl. Med.* 6:243ra86.
129. Lu P, *et al.* (2014) Three-dimensional structure of human gamma-secretase. *Nature.* 512:166–70.
130. Sun L, *et al.* (2015) Structural basis of human gamma-secretase assembly. *Proc. Natl. Acad. Sci. U. S. A.* 112:6003–8.
131. Li Y, *et al.* (2014) Structural biology of presenilin 1 complexes. *Mol. Neurodegener.* 9:59.

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