

# Presenilins in Memory, Alzheimer's Disease, and Therapy

## Minireview

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**Presenilins are considered to be the catalytic subunits of the  $\gamma$ -secretase complex and are therefore drug targets for Alzheimer's disease. They are also essential for the fine tuning of the immunological system and for memory and synaptic plasticity. Genetic ablation in the forebrain results in a progressive neurodegenerative process that is independent from A $\beta$  generation. The question arises as to what extent these observations should influence our thinking on the pathogenesis of Alzheimer's disease and on strategies to further develop  $\gamma$ -secretase inhibitors.**

Current Alzheimer's disease research focuses strongly on the amyloid generating and clearing processes. One hopes that the removal of the amyloid plaques, e.g., by vaccination, will stop or prevent the further neurodegenerative processes. Alternatively, inhibitors for either  $\beta$ - or presenilin/ $\gamma$ -secretase, the proteases that are responsible for the cleavage of the amyloid peptide (A $\beta$ ) from the amyloid precursor protein (APP), could prevent the accumulation of the plaques. For all three approaches, serious concerns have been raised about the feasibility or potential side effects, but presenilin/ $\gamma$ -secretase especially is a problem given its role in many crucial physiological functions. Moreover, Jie Shen and colleagues (Saura et al., 2004) demonstrated recently that complete loss of presenilin (PS) function in the forebrain leads to memory loss, synaptic dysfunction, and neurodegeneration—exactly what one aims to counteract in Alzheimer's disease. These results also suggest that, at least in the familial forms of Alzheimer's disease caused by presenilin mutations, neurodegenerative processes could be induced independently from abnormal A $\beta$  generation and possibly contribute to the severity of the disease process in this subgroup of patients. Before discussing the implications of the findings, some more background information is needed.

### **The Presenilins**

The presenilins PS1 and PS2 most likely provide the catalytic subunit of the large multiprotein complex called  $\gamma$ -secretase (De Strooper, 2003). Together with the three additional subunits (Nicastrin, Aph1, and Pen-2), they contribute a total of 18 hydrophobic transmembrane domains to the complex (Figure 1). These domains could create a microenvironment in the cell membrane that

allows the hydrolyzing of transmembrane domains of integral membrane proteins. Presenilin contains two aspartyl residues in its hydrophobic core that serve as a catalytic site. Missense mutations of these residues do not disturb the assembly of the  $\gamma$ -secretase complex but completely annihilate its proteolytic function (discussed in Nyabi et al., 2003). Some  $\gamma$ -secretase inhibitors bind directly to presenilins, and recent experiments show that structurally similar membrane bound proteases can be found throughout the animal kingdom, providing circumstantial but convincing evidence that the presenilins constitute a novel class of proteases. The most relevant physiological substrates for the presenilins are, without any doubt, the Notch proteins, large signaling receptors that are involved in a multitude of developmental pathways. Notch signaling starts with ligand binding followed by a conformational change in the Notch ectodomain and proteolytic cleavages eventually leading to the release of the Notch intracellular domain by  $\gamma$ -secretase. In addition, presenilins/ $\gamma$ -secretase cleave a number of other substrates, including N- and E-cadherin, LRP, Syndecan, Delta, Jagged, CD44, ErbB4, Nectin1 $\alpha$ , APP, and others (for an excellent review, see Kopan and Ilagan, 2004). The evidence is largely in vitro, and the physiological significance of these proteolytic processes remains unclear. One possibility is that presenilins are responsible for the clearance of transmembrane domains of proteins after they have accomplished their mission, thus serving as the “proteasome of the membrane” (Kopan and Ilagan, 2004). It is also possible, however, that presenilins are “molecular switches” in many different signaling pathways.

### **The Presenilins and Notch**

One major way to gain an understanding of the biological role of a protein is the genetic knockout approach in whole organisms (Table 1). Studies in flies, worms, and mice have all confirmed the crucial role of the *presenilins* (PS) in Notch signaling. For instance, while inactivation of *PS1* in mice (Hartmann et al., 1999; Shen et al., 1997; Wong et al., 1997) yields a phenotype that features only some aspects of deficient Notch-1 signaling (like disturbed somitogenesis), additional ablation of its homolog *PS2* (Donoviel et al., 1999; Herreman et al., 1999) results in a presumably “full” Notch-1 phenotype. Expression of Notch-1 signaling target genes like *Hes5* or *Delta-like* are altered in the mice (Handler et al., 2000; Donovan et al., 1999), confirming the deficiency at the molecular level. Recently published studies also shed some light on the function of presenilin in adulthood. *PS1<sup>+/-</sup>PS2<sup>-/-</sup>*-deficient mice (Tournoy et al., 2004; Qyang et al., 2004) survive in apparent healthy condition with only one PS1 allele until 6 months of age. They display about 40% reduction in  $\gamma$ -secretase activity in different organs. From 6 months on, however, seboreic keratosis and a spectacular autoimmune disorder develop with glomerulonephritis, vasculitis, and keratitis. The CD4/CD8 ratio of T lymphocytes is increased, probably reflecting deficient Notch signaling, which is critically involved in several differentiation steps of the

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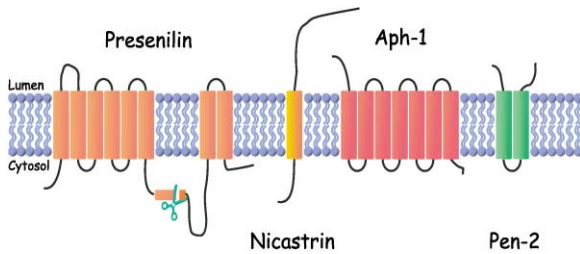


Figure 1. The Core of the  $\gamma$ -secretase Complex

T lymphocyte lineage. Qyang et al. (2004) found in their colony a myeloproliferative disorder dominating the phenotype of the mice. Strain differences or environmental factors could explain why the T cell lineage is affected in one model and the granulocyte-monocyte lineage is affected in another, but overall, the data demonstrate the essential functions of presenilins in different aspects of white blood cell differentiation. Other interesting mouse models have been generated using the Thy-1 promoter driving *PS1* expression to rescue the lethal deficiency in *PS1* knockout mice and resulting in more or less organ-specific knockouts. In (embryonic) kidney (Wang et al., 2003), absence of *PS1* and *PS2* results in altered nephrogenesis, likely because of deficient Notch signaling. In epidermis, loss of *PS1* (Xia et al., 2001) results in epidermal dysplasia and skin cancer.  $\beta$ -catenin accumulation was proposed as the molecular cause of this disease, but deficient Notch signaling could contribute to the phenotype as well (Nicolas et al., 2003).

Overall, it is clear that genetic deficiencies in presenilins affect a panoply of physiological functions, mainly via the Notch signaling pathway, but other pathways might contribute as well.

#### Presenilins and the Brain

Given the central role of presenilins in Alzheimer's disease, their function in the central nervous system is of crucial interest. Selective inactivation of *PS1* alone in the forebrain (*FB-PS1KO*) has been achieved by crossing floxed *PS1* gene mice (*PS1cKO*) with mice expressing Cre recombinase under the control of the  $\alpha$ CaMKII promoter (Feng et al., 2001; Yu et al., 2001). This results in the specific postnatal inactivation of *PS1* expression in excitatory neurons of the cortex, hippocampus, and amygdala (Table 1). The mice appeared remarkably normal: while they displayed a significant decrease in  $\gamma$ -secretase activity (with accumulation of APP carboxy-terminal fragments and decreased  $A\beta$  generation), no overt anatomical or functional deficits could be demonstrated. Interestingly, the expression of Notch downstream genes (*Hes 3* and *5*, *Delta-like 1*) was not affected in the brains of the *FB-PS1KO* mice. Only a discrete and mild impairment in some memory tests was seen. The overall picture emerging from these experiments seemed quite supportive for the concept of  $\gamma$ -secretase inhibitors. Apparently, adult brain (or more specifically, the excitatory neurons of the forebrain) can cope with decreased  $\gamma$ -secretase signaling, and the presence of *PS2* is sufficient to maintain Notch signaling. Obviously, this remained to be investigated. Thus, *FB-PS1KO* mice were generated on a *PS2*<sup>-/-</sup> background (Saura et al., 2004). Strikingly, this time a dramatic phenotype was observed, demonstrating that *PS2* is indeed largely able to compensate for

the lack of *PS1* in adult brain. More importantly, the results showed unequivocally the requirement of presenilins for the normal function of the neuronal synapse. Absence of presenilin expression in the neurons resulted in decreased NMDA receptor levels in the synaptic membrane and decreased  $\alpha$ CaMKII activity and altered *CREB/CBP* gene regulation in the neuron, resulting in impaired expression of *c-fos* and BDNF. The functional consequences are dramatic, with strongly impaired LTP, spatial and contextual memory deficits, and, after some time, massive loss of synapses, dendrites, and neurons. Remarkably, this neurodegeneration was accompanied by increased Tau phosphorylation, likely via activation of CDK5/p25. These results challenge the quite simple but preferred picture of the disease process in the familial forms of Alzheimer's disease caused by presenilin mutations. If these mutations cause partial loss of function (for a discussion, see Saura et al., 2004, and De Strooper, 2003), it is conceivable that, apart from the effects on  $A\beta$  generation, decreased Notch signaling and *CREB/CBP* activity could also contribute to the neurodegenerative process. This could explain why these patients display an earlier age of onset and a faster progression rate than the patients with APP mutations. A major question that needs further information is to what extent the synaptic changes are a consequence of the loss of  $\gamma$ -secretase function alone. Shen et al. (Saura et al., 2004) suggest that presenilins are needed for the correct transport and insertion of the affected NMDA receptors in the postsynaptic membrane. It should be noticed that similar molecular trafficking functions for presenilins have been proposed in the past but that the supporting evidence remains quite indirect. Decreased NMDA receptor signaling could explain the decreased  $\alpha$ CaMKII activity and the changes in LTP and synaptic transmission observed in the mice. Abnormal Notch signaling could also contribute to the overall phenotype: *CBP* contains a consensus sequence site for the CBF-1 (RBP-J $\kappa$ ) transcription factor, which is regulated by Notch (Saura et al., 2004). *CBP* is an essential cofactor for the transcription factor *CREB* and (genetic) deficiencies of these factors lead on their own to neurodegeneration (e.g., by decreased BDNF synthesis). Again, this link needs further confirmation but illustrates a recurrent theme in presenilin research, namely, the crucial contribution of deficient Notch signaling to any phenotype observed until now in *presenilin*-deficient animals.

#### Implications for Our Thinking on Alzheimer's Disease

The work with the brain-specific *presenilin* knockout mice makes clear how far away we still are from really understanding the neurodegenerative mechanisms operating in Alzheimer's disease. The question should be raised as to what extent the  $A\beta$  amyloid peptide alone is capable of inducing the whole process and to what extent additional factors are necessary or additive to the overall mechanism. The current publication (Saura et al., 2004) provides proof of the principle that, in familial Alzheimer's disease, both increased  $A\beta_{42}$  and loss of function of presenilin could independently contribute to the pathogenesis. The discussion of whether  $A\beta_{42}$  production reflects a "special gain" or a "partial loss" of function (Saura et al., 2004; De Strooper, 2003) is hereby opened again, but the issue also needs further exploration because it could shed light on some other

Table 1. Phenotypes in Partially or Fully Presenilin-Deficient Mice

Genotype	Viability and Overall Phenotype	Central Nervous System	References
General KO <i>PS1</i> <sup>-/-</sup>	lethal E17-P1; disturbed somitogenesis, skeleton abnormalities, angiogenesis deficits and hemorrhages, midline closure defects	underdeveloped ventricular zone, neuronal migration disorder (lissencephaly II)	1, 2, 3, 4, 5, 6
<i>PS2</i> <sup>-/-</sup>	normal; discrete lung fibrosis and hemorrhages	—	7, 8
<i>PS1</i> <sup>-/-</sup> <i>PS2</i> <sup>-/-</sup>	lethal E9.5; severe somite segmentation defects, heart and second branchial arch malformation, vascular defect in yolk sac	neural tube malformations	7, 8
<i>PS1</i> <sup>+/-</sup> <i>PS2</i> <sup>-/-</sup>	autoimmune disease with glomerulonephritis, keratitis, dermatitis, vasculitis, and seborrheic hyperkeratosis myeloproliferative disorder	—	9, 10
Tissue-specific KO <i>PS1cKO</i> × $\alpha$ CaMKII-Cre (FB- <i>PS1KO</i> )	normal; (KO in forebrain: hippocampus, amygdala, cortex)	mild impairment of spatial memory, subtle changes in neurogenesis	11, 12
<i>PS1cKO</i> × <i>PS2</i> <sup>-/-</sup> × $\alpha$ CaMKII-Cre (FB- <i>PS1KO</i> × <i>PS2</i> <sup>-/-</sup> )	neurodegeneration with neuronal loss and gliosis (KO in forebrain: hippocampus, amygdala, cortex)	severely impaired memory and synaptic plasticity (from 2 months on), synaptic loss, tau hyperphosphorylation	13
<i>PS1</i> <sup>-/-</sup> × <i>Thy1-PS1</i>	skin cancer; hyperplasia, keratosis, and neoplasia (KO in skin)	—	14
<i>PS1</i> <sup>-/-</sup> <i>PS2</i> <sup>-/-</sup> × <i>Thy1-PS1</i>	lethal P1; nephrogenesis deficits (KO in kidney)	—	15

1, Shen et al., 1997; 2, Wong et al., 1997; 3, Hartmann, et al., 1999; 4, Nakajima et al., 2003; 5, Takahashi et al., 2000; 6, Handler et al., 2000; 7, Donoviel et al., 1999; 8, Herreman et al., 1999; 9, Tournoy et al., 2004; 10, Qyang et al., 2004; 11, Yu et al., 2001; 12, Feng et al., 2001; 13, Saura et al., 2004; 14, Xia et al., 2001; 15, Wang et al., 2003.

long-outstanding issues, such as why transgenic mice with huge loads of A $\beta$  peptides in their brains do not develop massive neurodegeneration and why in some patients loads of A $\beta$  can be found in the brain without overt signs of ongoing neurodegenerative processes. One possibility of reconciling the “amyloid hypothesis” with the novel insights is to propose a “two hit” model for Alzheimer’s disease, in analogy with human cancer, implying that A $\beta$  is a burden for the neurons but becomes only really toxic once the neurons are under stress or are hit by additional insults. In the families with *presenilin* mutations, the two hits (abnormal A $\beta$ <sub>42</sub> generation and compromised synaptic function/neuronal survival) come from the same molecule; in other forms of the disease, the second hit could be basically any event that weakens the neurons’ ability to cope with stress. It still makes a lot of sense to try to block the production or increase the removal of A $\beta$  as a potential treatment for (sporadic) Alzheimer’s disease, but importantly, it also makes clear how important it is to focus a bit more on this hypothetical second hit and to look for the contributing factors that set the stage for the disease. The link from decreased synaptic activity to decreased neuronal survival via the CREB/CBP signaling pathway as outlined by Jie Shen and colleagues (Saura et al., 2004) could thereby become a central axis for further fruitful research.

#### **The End for Presenilins as Drug Targets?**

The accumulating data on the many biological roles of presenilins obviously also have implications for our thinking on  $\gamma$ -secretase as a viable drug target. Before making drastic decisions and putting aside presenilins/ $\gamma$ -secretase, one should gain some distance. Indeed, the genetic and usually complete deletion of a protein

might affect the organism in a very different way than the partial and changing modulation of its activity obtained by pharmacological intervention. For example, genetic inactivation of Glycogen synthase kinase-3 causes severe liver degeneration during embryogenesis, while LiCl, which inhibits this enzyme, is used for the treatment of manic depression. An even more striking example is 3-hydroxy-3-methylglutaryl-co-enzyme A reductase (HMG-coA). Millions of people worldwide are taking daily Statins to inhibit this enzyme, with the goal of lowering their cardiovascular risk. Genetic ablation of HMG-coA, however, results in an even more severe lethal phenotype than the one observed in *PS* knockout mice: no embryos survive beyond the blastocyst stage (Ohashi et al., 2003). Indeed, if the genetic experiments had the final word about the drug programs, we would never have had ACE inhibitors or Statins. Genetic experiments are therefore great for understanding the biological function of proteins, but their predictive value with regard to the pharmacology is limited, and only in vivo toxicity tests and eventually clinical trials can decide whether a drug has potential in the clinic or not. Furthermore, and in contrast to the genetic experiments, drug treatment can be stopped, and problems can be reversed. Knowing the biological function of the target allows, of course, a more directed monitoring of specific problems, and for this aspect of drug development, the information from basic research is certainly valuable. Finally, it should be pointed out that nobody aims to block  $\gamma$ -secretase completely for the treatment of Alzheimer’s disease.

#### **Opportunities for $\gamma$ -secretase Inhibitor Research**

The first issue to be considered when interpreting the *presenilin* genetic knockout data (Table 1) in the context

of drug development is that presenilins, in addition to their central role as catalytic subunits in the  $\gamma$ -secretase complex, also potentially have other functions. They have been implicated in  $\beta$ -catenin turnover, in apoptosis and  $\text{Ca}^{2+}$  homeostasis, in protein transport, and in the assembly of the  $\gamma$ -secretase complex. While it is not always clear (e.g., for the regulation of  $\text{Ca}^{2+}$ ) to what extent these functions are independent from their catalytic  $\gamma$ -secretase activity, the possibility that non- $\gamma$ -secretase functions of presenilins contribute to the overall PS knockout phenotype has to be taken into account. Obviously, inhibitors of  $\gamma$ -secretase will not necessarily affect such functions, such as, for example, the NMDA receptor transport deficits observed in the *FB-PS1KO*  $\times$  *PS2KO* mice or the  $\beta$ -catenin increases in the skin tumors in the *PS1KOxThy1-PS1* mice (Table 1). Nevertheless,  $\gamma$ -secretase activity seems to be the main function of presenilins. It remains a tantalizing question as to whether deficient Notch signaling is the real and main problem as it appear to be at first glance from the phenotypes of the mice (Table 1), or whether deficient processing of the many other presenilin substrates contributes to the overall phenotype as well (several aspects remain unstudied!). Comparison with the combined (quadruple) Notch1-4 knockout mice would be very helpful, but such mice are obviously not easy to breed. The issue is very important because it implies, at least in practical terms, that the problem of screening for appropriate  $\gamma$ -secretase inhibitors can probably be reduced to the problem of finding compounds that discriminate between APP and Notch processing. For this reason, it is unlikely that inhibitors specifically targeting the aspartyl residues in the catalytic site itself will ever make it in the clinic, unless careful experiments can define a therapeutic window in which  $\text{A}\beta$  generation can sufficiently be inhibited without causing major side effects. On the other hand, circumstantial evidence in the literature indicates that the  $\gamma$ -secretase complex "sees" Notch and APP differently. Targeting selectively the putative exosites where APP or Notch bind to the complex is theoretically a possibility.

An alternative approach could take advantage of the noteworthy "relaxed" specificity of the  $\gamma$ -secretase. This enzyme can cleave the transmembrane domains of its different substrates at several positions, a feature that is not well understood. However, as has been demonstrated with a selective subgroup of nonsteroidal anti-inflammatory drugs (NSAIDs), it is possible to manipulate this property and to generate small shifts in the preferred cleavage sites without changing the overall processing of, for example, Notch (Weggen et al., 2001). With these NSAIDs, it is possible to promote the generation of shorter versions of the  $\text{A}\beta$  peptide that are believed to be less prone to amyloid formation. Importantly, clinical mutations in the presenilins also induce such small shifts but in the opposite direction, thus inducing the generation of the longer, strongly amyloid-prone  $\text{A}\beta_{42}$  peptide. At least the experiments with NSAIDs give proof of the principle that it is possible to manipulate  $\gamma$ -secretase activity without inducing massive toxicity. Also something to be considered is the fact that no good treatments for Alzheimer's disease are available and that the number of alternative drug targets at this moment is

extremely limited. We had better think twice before dropping the presenilins from our drug target basket.

In conclusion, it is clear that the genetic studies on *presenilins* have taught us that more research "out of the box" in the Alzheimer's field is needed. We should try to gain a much better understanding of which factors in the brain contribute to the toxicity of  $\text{A}\beta$  and how this leads to disease. This is required if we want to find additional drug targets outside the strict borders of the "amyloid cascade hypothesis" and to increase our chances of finding cures for this terrible disease.

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