EMBO reports

talking point

Loss-of-function presenilin mutations in Alzheimer disease

Talking Point on the role of presenilin mutations in Alzheimer disease

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Presenilin mutations are the main cause of familial Alzheimer disease. From a genetic point of view, these mutations seem to result in a gain of toxic function; however, biochemically, they result in a partial loss of function in the γ-secretase complex, which affects several downstream signalling pathways. Consequently, the current genetic terminology is misleading. In fact, the available data indicate that several clinical presenilin mutations also lead to a decrease in amyloid precursor protein-derived amyloid β-peptide generation, further implying that presenilin mutations are indeed loss-of-function mutations. The loss of function of presenilin causes incomplete digestion of the amyloid β-peptide and might contribute to an increased vulnerability of the brain, thereby explaining the early onset of the inherited form of Alzheimer disease. In this review, I evaluate the implications of this model for the amyloid-cascade hypothesis and for the efficacy of presenilin/γ-secretase as a drug target.

Keywords: Alzheimer; gain or loss of function; presenilin

EMBO reports (2007) **8,** 141–146. doi:10.1038/sj.embor.7400897

Introduction

Alzheimer disease (AD) is a common and disabling disorder. Its incidence increases exponentially with age and therefore it is a significant public-health concern. Only symptomatic treatment is currently available; however, there is a legitimate hope for a cure thanks to the tremendous progress that is being made in understanding the molecular pathogenesis of the disease. The dominant theory in the field is the 'amyloid-cascade hypothesis', which links abnormal amyloid precipitates in the brain with neuronal dysfunction, the induction of tangles and dementia (Hardy & Higgins, 1992). The logical correlate is that drugs that either block amyloid β-peptide (Aβ) generation or increase its clearance, for example by vaccination, will cure or halt the progression of AD. Although a great deal of evidence supports this relatively simple and straightforward concept (Annaert & De Strooper, 2002; Hardy & Selkoe, 2002), there is no proof that this theory has clinical relevance. As a result, the amyloid-cascade hypothesis has received some criticism in recent years. The current discussion revolves around presenilin 1 (encoded by *PSEN1*), and the extent to which gain or loss of function of this gene (Fig 1) contributes to the pathological spectrum of early-onset familial AD. Presenilin 1—along with the closely related presenilin 2—is the catalytic component of the γ-secretase enzyme, which cleaves the amyloid precursor protein (APP) into Aβs of varying lengths (De Strooper *et al*, 1998). The discussion is complicated by the fact that researchers use different strategies to evaluate *PSEN* function and, although evidence indicates that *PSEN* mutations result in changes in Aβ generation in accordance with the amyloid-cascade hypothesis (Scheuner *et al*, 1996), the complete loss of *Psen* function in the brains of mice results in neurodegeneration in the total absence of Aβ generation (Saura *et al*, 2004). This latter research has led to the theory that Aβ generation is not necessary for the development of AD.

Clinical mutations in *PSEN1* cause AD

So far, more than 150 familial AD-causing mutations in *PSEN1* have been identified, approximately 10 additional mutations have been found in the homologous gene *PSEN2* and 25 mutations have been identified in the *APP* gene (http://www.molgen.ua.ac.be/ADMutations). The study of *PSEN1* is therefore crucial for understanding the pathogenesis of familial AD.

Most mutations in *PSEN1* are simple missense mutations that result in single amino-acid substitutions in presenilin 1. Some are more complex, for example, small deletions, insertions or splice mutations. The most severe mutation in *PSEN1* is a donor–acceptor splice mutation that causes two amino-acid substitutions and an in-frame deletion of exon 9 (Fig 1). Significantly, however, the biochemical consequences of these mutations for γ-secretase assembly are limited (Bentahir *et al*, 2006; Steiner *et al*, 1999). Although as many as one-third of the 467 amino acids in the open-reading frame of presenilin 1 are affected by disease-causing mutations, a truncation or absence of the protein has never been observed, indicating that haploinsufficiency does not cause AD. Rather, at first glance and from a strictly genetic perspective, these different clinical mutations all seem to lead to a specific gain of toxic function for

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Submitted 12 September 2006; accepted 13 November 2006

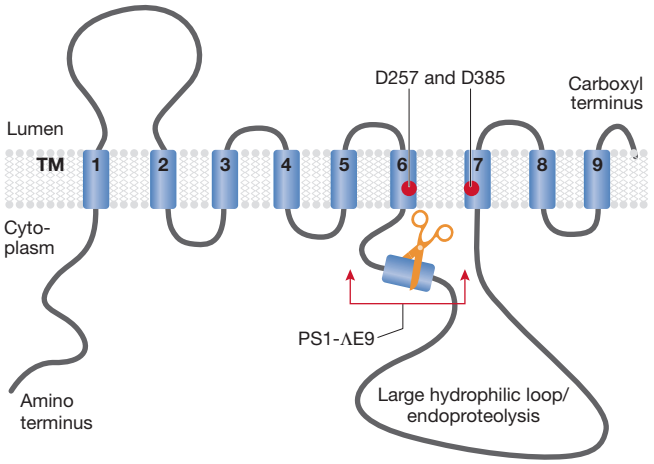

Fig 1 | Nine-transmembrane topology for presenilin. Presenilin is schematically represented. Two aspartate residues in transmembrane domains (TMs) 6 and 7 constituting the catalytic site are indicated. The Δexon9 mutation (PS1–DE9) deletes the indicated region of the protein.

PSEN1. Investigations over several years, however, have not succeeded in translating this genetic concept into molecular terms—that is, explaining how the mutations scattered over presenilin 1 all cause a similar gain of toxic function in the protein.

Mutations in either presenilin or APP consistently increase the relative ratio between the long (Aβ42) and short (Aβ40) amyloid peptides (Aβ42/Aβ40; Borchelt *et al*, 1996; Scheuner *et al*, 1996). Given that inactivation of *Psen1* and *Psen2* completely prevents Aβ generation (Herreman *et al*, 2000; Zhang *et al*, 2000), this increase can indeed be explained as a gain of toxic function. However, the change in ratio can also be the consequence of a partial loss of Aβ40 generation, as is the case with several *PSEN* mutations discussed in detail below. Indeed, several authors have challenged the dominant gain-of-toxic-function hypothesis over the years. First, wild-type human *PSEN1* can effectively rescue the loss of its suppressor of Notch-family member lin-12 (*sel-12*) homologue in *Caenorhabditis elegans*, whereas a mutated *PSEN1* is less effective or not effective at all (Baumeister *et al*, 1997; Levitan *et al*, 1996). Second, Shen and co-workers have shown that a total loss of *Psen* function in the forebrain of mice causes neurodegenerative disease in the absence of Aβ (Saura *et al*, 2004). Third, several groups have reported that specific loss of *Psen1* in the mouse forebrain affects particular aspects of memory (Feng *et al*, 2001; Yu *et al*, 2001). Both neurodegeneration and memory deficits are important features of AD; however, it might be dangerous to extrapolate these observations to human pathology. Accordingly, it is difficult to correlate the total loss of four *Psen* alleles in a mouse model (Saura *et al*, 2004) with the relatively mild single mutation of one *PSEN* allele in familial AD patients. Indeed, neurodegenerative phenotypes have not been observed in animal models with only one allele inactivated (*Psen1*$^{+/-}$ or *Psen2*$^{+/-}$; for a more detailed overview of the different *Psen*-knockout mouse models, see Marjaux *et al*, 2004). Furthermore, it is unclear whether the memory deficits in mice with a forebrain-specific *Psen1*-knockout can really be compared with the memory deficits in patients with AD. In this regard, some of the memory deficits that result from APP overexpression in AD mouse models can be alleviated by *Psen1* inactivation (Dewachter *et al*, 2002; Saura *et al*, 2005). Fourth, in the past few years, some studies have indicated that genuine loss-of-function *PSEN1* mutations could be involved in forms of frontotemporal dementia without the involvement of Aβ (Amtul *et al*, 2002; Dermaut *et al*, 2004; Raux *et al*, 2000). However, formal genetic or molecular proof that these mutations are responsible for the neurodegenerative process in these patients has not been provided. In fact, an additional mutation in the progranulin gene (Baker *et al*, 2006; Cruts *et al*, 2006) in a patient with the presenilin 1 Arg352 insertion (Boeve *et al*, 2006) is probably the cause of the dementia, which implies that, at least in this case, the mutation in presenilin 1 is a polymorphism. Finally, promotor polymorphisms in the *PSEN1* gene that decrease its expression contribute to the risk of early-onset AD (Theuns *et al*, 2000); however, whether these affect amyloid generation is not yet known.

In conclusion, although the current research clearly indicates that presenilin 1 is important for maintaining the integrity of the brain, it is less clear whether the severe deficits in homozygous loss-of-function mouse models are relevant to the pathology in human patients. Furthermore, *PSEN1* deficiency is unlikely to contribute to the disease process in patients with APP mutations, which implies that the effects of loss of *PSEN1* function on APP processing are crucial for our understanding of the pathogenesis of AD. I do not, however, exclude the possibility that partial dysfunction of *PSEN1*—for example, in the Notch signalling pathway that modulates neurite outgrowth and brain repair—makes the brain more prone to Aβ toxicity. This would fit with the previously proposed 'two-hit' model for AD (Marjaux *et al*, 2004), and would also explain why familial AD generally strikes earlier and is more aggressive than sporadic AD.

Presenilin as part of the γ-secretase complex

Presenilin provides the catalytic core of γ-secretase, which removes short transmembrane protein fragments from the cell membrane (De Strooper, 2003). γ-secretase is a highly hydrophobic complex (Fig 2) consisting of at least three additional subunits—nicastrin, Aph1 and Pen2—which, together with presenilin, form a barrel-like structure in the membrane (Lazarov *et al*, 2006). Water, which is necessary for the catalytic activity of the complex, is present in this structure (Tolia *et al*, 2006). Considering the fact that there are two *PSEN* genes and two *APH* genes, at least four different complexes with potentially different biological functions (Serneels *et al*, 2005) could co-exist in cells and tissues (Hébert *et al*, 2004; Shirotani *et al*, 2004). The current models indicate that the aminopeptidase-like domain of nicastrin, which functions as an exosite on the protease, provides a docking site for γ-secretase substrates (Shah *et al*, 2005). Significantly, more than 30 different substrates have been identified, including APP. Following sequential cleavage of APP by the β-secretases and γ-secretases, the major proteolytic products—Aβ and the APP intracellular domain (AICD)—are released extracellularly and intracellularly, respectively. Although it has been frequently proposed that AICD is a signalling molecule similar to the Notch intracellular domain (NICD; Hébert *et al*, 2006; Kopan & Ilagan, 2004; Marambaud *et al*, 2003), this has not been rigorously proven.

Sequence determination of the carboxyl terminus of Aβ and the amino terminus of AICD has revealed heterogeneity (Fig 3). There are two γ-cleavage products, which end at either residue Val40 (Aβ40) or residue Ala42 (Aβ42). The AICD peptide starts at Val51 or Met52 and

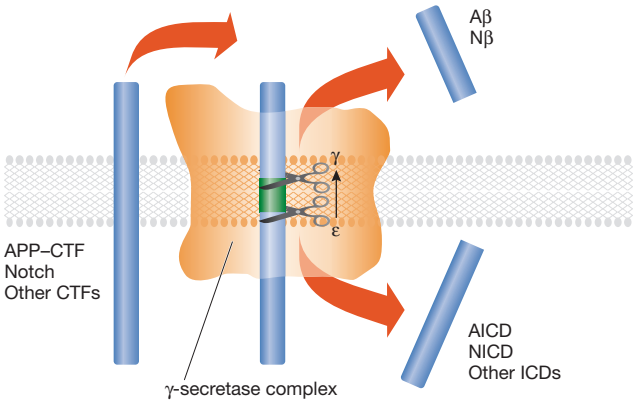

Fig 2 | The γ-secretase. Schematic drawing of the 'barrel' structure of the γ-secretase (orange). The arrow indicates progressive ε-to-γ cleavage of the substrate (blue bars). The possibility of unfolding of the substrate is discussed in the main text. Aβ, amyloid β-peptide; AICD, APP intracellular domain; APP, amyloid precursor protein; CTF, carboxy-terminal fragment; ICD, intracellular domain; Nβ, Notch β-like fragment; NICD, Notch intracellular domain.

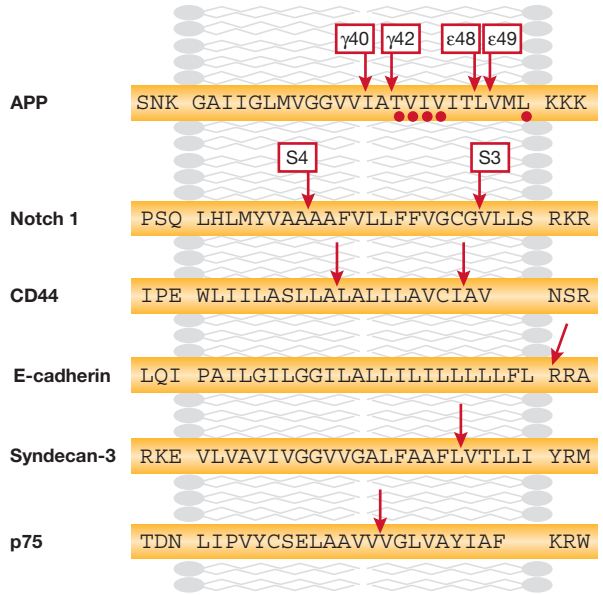

Fig 3 | Cleavage sites of selected γ-secretase substrates. The transmembrane sequences of several substrates of the γ-secretase are indicated. Red arrows indicate cleavage sites deduced from amino-terminal and carboxy-terminal sequencing. APP, amyloid precursor protein.

these cleavages are recognized as the ε-site cleavage products (Fig 3). Similar dual cleavages have been identified for Notch (Okochi *et al*, 2002) and CD44 (Lammich *et al*, 2002). Evidence indicates that γ-secretase cuts APP initially at the ε-site and then progressively removes C-terminal residues until the γ-cleavage site has been reached (Fig 2; Kakuda *et al*, 2006). It is likely that the hydrophobicity of the remaining peptide is then sufficiently reduced to facilitate its release into the extracellular medium. This model would explain the following observations: neither AICD nor NICD peptide fragments that extend N-terminally beyond the ε-site have been detected (Chandu *et al*, 2006; Kakuda *et al*, 2006); longer Aβs—Aβ43 up to Aβ49—can be detected in cell extracts (Qi-Takahara *et al*, 2005; Yagishita *et al*, 2006; Zhao *et al*, 2005); tryptophan mutations introduced between the ε- and γ-cleavage sites in APP block cleavage at the γ-site (Sato *et al*, 2005); and a product–precursor relationship has been detected between long and short forms of Aβ (Qi-Takahara *et al*, 2005; Yagishita *et al*, 2006).

Alternative models are more complicated, invoking, for example, additional catalytic sites in γ-secretase or carboxypeptidases that act after the initial ε-site cleavage. It remains unclear how substrates are progressively cleaved in the same catalytic site. It is possible that the α-helix of putative substrates unfolds after ε-site cleavage, which, in turn, advances the next bound peptide towards the catalytic site for further cleavage. The consecutive cleavage of APP could provide an explanation for how loss-of-function mutations in *PSEN1* might result in decreased Aβ generation and simultaneous increased production of long Aβ (Bentahir *et al*, 2006; Kumar-Singh *et al*, 2006; Qi *et al*, 2003).

PSEN mutations are loss-of-function mutations

The first *in vivo* evidence that *PSEN* mutations cause a loss of Notch signalling was provided in *C. elegans* (Baumeister *et al*, 1997; Levitan *et al*, 1996). However, two follow-up reports in mice did not correlate with these observations (Davis *et al*, 1998; Qian *et al*, 1998). The discrepancy in these studies might be explained by the fact that in the mouse studies, the authors used a *Psen1* Ala246Glu mutation under a heterologous promotor in their rescue experiments and observed only a partial rescue of the phenotype. Furthermore, in cell-based assays, this mutation resulted in only a 20% reduction in the cleavage of Notch (Bentahir *et al*, 2006). These rescue experiments have not shed light on *PSEN* loss-of-function mutations. Knock-in mutations provide a better way to address this question. Several mutant mice have been described with relatively mild phenotypes (Guo *et al*, 1999; Nakano *et al*, 1999; Siman *et al*, 2000). Memory deficits were observed in one mouse model containing two diseased alleles, which were rescued in heterozygous mice indicating a loss-of-function phenotype (Wang *et al*, 2004). Experiments in cell lines unequivocally confirm that *PSEN* mutants decrease the cleavage of Notch, syndecan and N-cadherin, and this can probably be extended to other substrates (Baki *et al*, 2001; Bentahir *et al*, 2006; Schroeter *et al*, 2003; Song *et al*, 1999). Therefore, *PSEN* mutations result in a loss of function of the γ-secretase. In fact, a wealth of additional experiments examining *PSEN* function in protein trafficking, apoptosis, autophagy, calcium homeostasis, β-catenin turnover, regulation of kinase pathways and tau phosphorylation all support the loss-of-function interpretation. These topics are not discussed further within the scope of this review.

Loss of γ-secretase changes the Aβ42/Aβ40 ratio

The effects of *PSEN* clinical mutations on APP processing have mostly been investigated by analysing the Aβ42/Aβ40 ratio; this permits the normalization of differences in APP or presenilin expression in different cell lines and is considered to be a prominent factor for disease progression in familial AD patients (Borchelt *et al*, 1996; Duff *et al*, 1996; Scheuner *et al*, 1996). More than 10 years ago, Jarrett and Lansbury described Aβ42 as a 'nucleation' factor, which notably accelerates the aggregation of Aβ into amyloid *in vitro* (Jarrett

& Lansbury, 1993). Direct evidence for the importance of Aβ42 in AD came from a biochemical analysis of the APP Val717Ile clinical mutation (Goate *et al*, 1991). This mutation and several others all cause an increase in the generation of Aβ42 relative to Aβ40 (Suzuki *et al*, 1994). Finally, Aβ42, although generated by neurons at a tenfold lower rate than Aβ40, is the main component of amyloid plaques in the brains of AD patients (Iwatsubo *et al*, 1994).

Given the enormous differences in the biophysical properties of the Aβ40 and Aβ42 peptides, it is surprising that the AD field has spent so much time focusing on the quantitative rather than the qualitative aspects of this increased ratio. For instance, the combination of a mutant *Psen1* allele with a *Psen1*-null allele causes accelerated amyloidosis, whereas the combination of the same mutant with a wild-type *Psen1* allele is protective, even with an absolute increase in γ-secretase activity (Wang *et al*, 2006). Furthermore, expression of Aβ42—but not Aβ40—alone is sufficient to cause amyloidosis in transgenic mice (McGowan *et al*, 2005).

The effects of *PSEN* clinical mutations on APP processing were recently re-evaluated in cell-culture systems. Mutants were either expressed in a *Psen*-negative background (Bentahir *et al*, 2006; Walker *et al*, 2005) or stably transfected (Kumar-Singh *et al*, 2006) and the levels of expression were carefully monitored. These studies analysed the absolute levels of Aβ40 and Aβ42, and, importantly, the accumulation of APP C-terminal fragments, which are direct substrates for γ-secretase. An increase in Aβ42/Aβ40 was confirmed; however, this was due to a decrease in Aβ40 peptide levels in several mutants. Importantly, all cell lines accumulated APP C-terminal fragments and showed decreased generation of the cytoplasmic AICD (Bentahir *et al*, 2006; Kumar-Singh *et al*, 2006; Walker *et al*, 2005; Wiley *et al*, 2005), thereby establishing that *PSEN* mutations result in a loss of γ-secretase cleavage of APP. This apparently translates into an 'incomplete digestion' of the APP substrate, generating fewer but longer Aβs (Qi *et al*, 2003; Yagishita *et al*, 2006). It is therefore clear that biochemical loss of function of presenilin can cause AD. The confusion with the genetic gain-of-toxic-function view can, however, be resolved because the loss-of-*Psen* mutations act indirectly in the disease process, causing a gain of toxic function of the *APP* gene by the incomplete digestion of Aβ.

Implications for the amyloid-cascade hypothesis

Since the original amyloid-cascade hypothesis for AD was put forward (Hardy & Higgins, 1992), many modifications and refinements have been proposed to incorporate new observations and to resolve apparent conflicts. For example, no absolute relationship exists between amyloid load in the brain and the clinical manifestation of AD symptoms in humans (Price & Morris, 1999) or mice (Games *et al*, 1995). This has led to the concept of Aβ-derived diffusible ligands (Lambert *et al*, 1998) or 'soluble toxic oligomers' (Glabe, 2006; Lambert *et al*, 1998; Walsh *et al*, 2002). These Aβ oligomers are intermediary forms between free soluble Aβs and insoluble amyloid fibres, and seem to be toxic both *in vitro* and *in vivo*. Although the molecular nature of these oligomers remains elusive, they have been isolated from transfected Chinese hamster ovary cells (Walsh *et al*, 2002) and as a 56-kDa oligomer from transgenic mouse brains (Lesne *et al*, 2006). The extent to which *PSEN1* mutations generate mixtures of Aβs that are more prone to form toxic oligomers remains to be investigated; however, this concept could explain cases of AD in which smaller amounts of Aβ are generated. More research is needed to determine the biophysical and biochemical properties of this species. However, we are clearly moving away from amyloid plaques and fibrils towards a more functional definition of Aβ toxicity, and it might be appropriate to indicate this paradigm shift by addressing the 'Aβ-tangle cascade' hypothesis in the future.

There are important implications for therapeutic approaches to AD. It is now essential to investigate how different Aβ-peptide species contribute to the generation, stability and toxic properties of the oligomers. The relative combination of these peptides could be much more important than the total load of Aβ in the brain. Inhibiting Aβ generation by β- or γ-secretase inhibitors might still be a good idea, considering the fact that a reduction of the overall load of free peptide will probably influence the balance between Aβ in free, oligomeric and amyloid fibril conformations; however, it is becoming increasingly crucial to elucidate the extent to which the last of these is in equilibrium with the toxic oligomer conformation. γ-secretase inhibitors might provide additional possibilities because some have been shown to modulate the activity of the enzyme by shifting the spectrum of Aβs to shorter, probably more soluble forms, like the non-steroidal anti-inflammatory drugs (Weggen *et al*, 2003), or to longer membrane-bound peptides, such as the γ-secretase inhibitor DAPT (Qi-Takahara *et al*, 2005; Yagishita *et al*, 2006). For non-catalytic site-directed inhibitors of γ-secretase, the observed paradoxical increases in Aβ42 might be explained by this vision. However, before testing these drugs in the clinic, it will be important to investigate the fate of the peptides in biological systems, and to determine how the other forms of Aβ contribute to the generation and stabilization of Aβ oligomers.

ACKNOWLEDGEMENTS

The author sincerely thanks A. Thathiah, K. Devriendt, E. Legius, T. Golde, M. Hutton and B. Boeve for interesting discussions. The author is supported by a Freedom to Discover grant (Bristol Myers Squib), a Pioneer award (Alzheimer's Association), the Fund for Scientific Research (Flanders), K.U.Leuven (GOA), the European Union (APOPIS: LSHM-CT-2003-503330) and the Federal Office for Scientific Affairs (Belgium).

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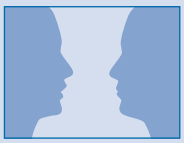

For more discussion on this topic, see also
Hardy J (2007) Putting presenilins centre stage. This issue p134.
Wolfe MS (2007) When loss is gain: reduced presenilin proteolytic function leads to increased Aβ42/Aβ40. This issue p136.