When loss is gain: reduced presenilin proteolytic function leads to increased A β 42/A β 40

Talking Point on the role of presenilin mutations in Alzheimer disease

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More than 100 missense mutations in presenilin 1 and 2 are associated with early-onset dominant Alzheimer disease. These proteins span the membrane several times and are ostensibly the catalytic component of the γ -secretase complex, which is responsible for producing the amyloid β -peptide (A β) that deposits in the Alzheimer brain. A common outcome of Alzheimer-associated presenilin mutations is an increase in the ratio of the more aggregation-prone 42-residue form of A β to the 40-residue variant, which is often referred to as a presenilin 'gain of function'. An apparent paradox is that most of these mutant presenilins have reduced proteolytic efficiency, which forms part of the counter argument that presenilin 'loss of function' can cause the neuronal dysfunction and death that lead to the disease. In this review, a unifying hypothesis is presented that puts forward a biochemical mechanism by which slower less-efficient forms of the protease can result in a greater proportion of 42-residue A β .

Keywords: amyloid β -peptide; enzyme mechanism; Notch receptor; pathogenesis; protease

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Introduction

A century ago, Alois Alzheimer first described the neurological disease that bears his name. He detailed two characteristic pathological features that are observed post mortem: amyloid plaques, and neurofibrillary tangles in the cerebral cortex and limbic system. Many decades passed before the biochemical components of these two features were discovered. The plaques are composed primarily of the 39–43-residue amyloid β -peptide (A β), and the tangles include filaments of the otherwise microtubule-associated protein tau (Wolfe, 2006). A β is released from the amyloid precursor protein (APP) by the sequential action of β - and γ -secretases, with the latter cutting relatively heterogeneously (Esler & Wolfe, 2001). Most of the A β produced by γ -secretase is the 40-residue form (A β 40);

however, the major A β species deposited in the plaques is the 42-residue variant (A β 42), although this peptide represents only 5–10% of all A β produced. In 1988, the *APP* gene was identified and found to reside on chromosome 21, which is triplicated in Down syndrome (trisomy 21). Indeed, most Down syndrome patients manifest Alzheimer disease (AD) by the age of 50, and post-mortem analyses of those who die young show diffuse intraneuronal deposits of A β in the absence of any tau pathology, suggesting that A β deposition is an early event in AD.

APP was the first gene to be associated with early-onset dominant AD, and the responsible mutations are located in and around the A β region of the encoded type I integral membrane glycoprotein. These mutations were soon determined to alter the amount of A β produced, the ratio of A β 42 to A β 40 or the amino-acid sequence of A β (Selkoe, 1994). Mutations near the β -secretase cleavage site make APP a better substrate for this enzyme and lead to increased production of all forms of A β . Mutations near the γ -secretase cleavage site lead to increases in the more aggregation-prone A β 42 relative to A β 40, and mutations in the A β region itself change the biophysical properties of the peptide to render it more likely to aggregate. These findings provided the first compelling evidence that A β might be involved in the pathogenesis of AD rather than simply being a marker. The recent discovery of an extra copy of the *APP* gene in familial AD (Rovelet-Lecrux *et al*, 2006) provides further support that increased A β production can cause the disease.

In 1995, mutations in the presenilins—PS1 and PS2—were also linked with early-onset familial AD (Levy-Lahad *et al*, 1995; Rogaev *et al*, 1995; Sherrington *et al*, 1995). The multi-pass membrane proteins encoded by these genes bore little resemblance to any other proteins known at the time, and their biochemical function was a complete mystery. However, the AD-associated mutations were soon determined to increase the ratio of A β 42 to A β 40 (A β 42/A β 40) in mice and humans (Borchelt *et al*, 1996; Citron *et al*, 1997; Scheuner *et al*, 1996), indicating that presenilins might modify the way in which γ -secretase cuts APP. Subsequently, the knockout of PS1 in mice was found to markedly reduce γ -secretase cleavage of APP (De Strooper *et al*, 1998), and follow-up studies showed that the knockout of both PS1 and PS2 eliminated γ -secretase cleavage completely (Herreman *et al*, 2000; Zhang *et al*, 2000). Presenilins

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are therefore essential for this proteolytic function. Parallel studies revealed that peptide-substrate mimics with classical aspartyl protease-inhibitory motifs could inhibit γ -secretase, suggesting that the enzyme belongs to this category of proteases (Wolfe *et al*, 1999a). This led to the identification of two conserved transmembrane aspartates in presenilin that are essential for γ -secretase activity, and the idea that presenilin is the catalytic component of the enzyme (Wolfe *et al*, 1999b,c). This hypothesis was soon confirmed through affinity labelling with transition-state analogue inhibitors (Esler *et al*, 2000; Li *et al*, 2000). More recently, three presenilin-associated co-factors for γ -secretase—the membrane proteins nicastrin (NCT), anterior pharynx-defective phenotype 1 (Aph1) and PS enhancer 2 (Pen2)—were discovered (Edbauer *et al*, 2003; Francis *et al*, 2002; Goutte *et al*, 2002; Kimberly *et al*, 2003; Takasugi *et al*, 2003; Yu *et al*, 2000), and purification of what is now known as the γ -secretase complex proved that presenilin and its three co-factors are necessary and sufficient for γ -secretase activity (Fraering *et al*, 2004). Furthermore, the discovery that presenilin homologues, such as signal peptide peptidase, apparently have proteolytic activity alone (Weihsen *et al*, 2002), without the need for other protein factors, cemented the concept of presenilin as the catalytic component of the γ -secretase complex. The implications for how presenilin mutations cause familial AD were clear: the mutations occur in the catalytic subunit of the protease responsible for determining the length of A β peptides (see Box). Nevertheless, it should be pointed out that presenilin also has nonproteolytic functions (Baki *et al*, 2004; Huppert *et al*, 2005; Kang *et al*, 2002), the disruption of which might also contribute to familial AD pathogenesis.

Concomitantly, however, it became clear that presenilin and γ -secretase have other substrates besides APP. After interacting with its cognate ligands, the Notch receptor undergoes a series of proteolytic events, in a manner similar to APP, ultimately releasing the intracellular domain that mediates the expression of genes controlling many types of cellular differentiation. We now know that the same γ -secretase cuts the transmembrane domain of the APP and Notch families of proteins, as well as a long list of other type I integral membrane proteins, including neuregulin and its receptor ErbB4, E-cadherins and N-cadherins, CD44, LDL-receptor-related protein (LRP), nectin-1 and growth hormone receptor. Cleavage of some of these substrates apparently has a role in cell signalling, but other cleavage events might simply be a means of clearing out membrane-bound stubs after ectodomain shedding (Kopan & Ilagan, 2004). The discovery of a new substrate has often led to suggestions that alterations in its proteolysis by presenilin mutations might underlie or contribute to AD. However, these speculations do not explain why APP mutations that change the production and/or properties of A β also cause the disease. This would require separate mechanisms of pathogenesis for presenilin and APP mutations, which is unlikely given that the former produces A β ; furthermore, it does so at the crucial site that determines the length of A β and its aggregation properties.

In parallel with the discovery of presenilin as a protease that cleaves APP and Notch, AD-causing mutations in presenilin were found to have reduced proteolytic function. Yankner and colleagues first showed this effect with a range of mutant presenilins by using a Notch-based luciferase-reporter assay (Song *et al*, 1999). Several other groups have since noted this phenomenon (for example, Lewis *et al*, 2000), with the most recent being De Strooper and colleagues who showed that mutations in presenilin reduced its

Table 1 | Summary of evidence supporting increased amyloid β -peptide (A β) or A β 42/A β 40 in the pathogenesis of presenilin mutations

Alzheimer disease (AD)-causing amyloid precursor protein (APP) mutations increase A β or A β 42/A β 40.
Triplification of the APP gene, either alone or with all of chromosome 21, leads to AD.
Brain A β 42 deposits are observed early and presymptotically in trisomy 21.
Presenilin is the catalytic component of γ -secretase, produces A β and determines the carboxy-terminal length of A β peptides.
AD-causing presenilin mutations increase A β 42/A β 40; the fact that presenilin and APP mutations cause AD by a common mechanism is parsimonious and logical, as presenilin cleaves APP to produce A β .
No AD-associated mutations have been found in any other γ -secretase substrate besides APP.
Among more than 100 AD-causing mutations in presenilins, complete loss of function of a single allele has not been found.
The Ihara model of processive proteolysis of APP by γ -secretase points to a biochemical mechanism by which a less-efficient protease (reduction of function) can lead to an increase in A β 42/A β 40 (gain of function).

proteolytic function towards several different substrates (Bentahir *et al*, 2006). These findings raise an apparent paradox, in which AD-causing disease mutations cause both a 'gain of function'—an increase in A β 42/A β 40—and a 'loss of function'—a decrease in proteolytic activity. These seemingly opposing effects have elicited considerable debate over how the presenilin mutations cause AD, with some researchers suggesting that reducing A β production with candidate therapeutics might even exacerbate or cause the disease (for further details, see the online discussion at <http://www.alzforum.org>). Below, I offer a synthesis of these two phenomena, which, at first glance, seem to be antithetical.

To appreciate the resolution of this purported paradox, it should initially be noted that the presenilin-containing γ -secretase complex cuts the transmembrane domain of APP, and other substrates, in at least two positions: the γ -site that produces the carboxyl terminus of A β and the ϵ -site further downstream that produces the amino terminus of the APP intracellular domain (AICD; Fig 1; Weidemann *et al*, 2002). Cleavage at the γ -site is heterogeneous, producing A β peptides of 39–43 residues, whereas the cut at the ϵ -site produces almost exclusively a 50-residue AICD. The same phenomenon occurs with Notch, involving heterogeneous cleavage in the middle of the transmembrane domain (the S4 site) and homogeneous cleavage further downstream (at the S3 site; Okochi *et al*, 2002). Interestingly, proteolysis at these two sites is affected by AD-causing mutations in APP and the presenilins, which lead to an increase in the proportion of A β 42 relative to A β 40 along with an increase in a new 51-residue AICD relative to the 50-residue product (Sato *et al*, 2003). These two proteolytic events are therefore not completely independent: a change in the cleavage site in one correlates with a change in the cleavage site of the other. However, it should be pointed out that in one study, several artificial mutations of Lys 166 in PS1 increased A β 42 production without affecting AICD levels (Moehlmann *et al*, 2002), and in another report, inhibition of endocytosis altered AICD formation without changing A β 42/A β 40 (Fukumori *et al*, 2006).

Recent evidence from Ihara and colleagues indicates that the ϵ -cleavage event might occur before proteolysis at the γ -site. First,

Fig 1 | Ihara model of processive proteolysis of the amyloid precursor protein transmembrane domain by γ -secretase, beginning at the ϵ -cleavage site and cleaving every three residues. This model explains how reduction of proteolytic function owing to presenilin mutations might lower amyloid β -peptide (A β) production but increase the ratio of A β 42 to A β 40. Longer forms of A β , with more of the hydrophobic transmembrane domain, might be more likely to be retained in the active site of the protease, whereas the shorter forms are more likely to be released. Less catalytically efficient γ -secretase complexes would allow more time for the release of longer A β peptides. In addition, Alzheimer disease-causing presenilin mutations shift the initial ϵ -cleavage site to produce more A β 48, which would lead to A β 42. AICD, APP intracellular domain; APP, amyloid precursor protein.

analysis of intracellular A β reveals a small but significant amount of longer forms of this peptide, up to A β 49, which is the proteolytic counterpart to the 50-residue AICD (Qi-Takahara *et al*, 2005); by contrast, longer AICDs, for example AICD counterparts to A β 40 or A β 42, have not been detected. Second, expression of A β 49 leads to the secretion of A β 40 and A β 42 in the same proportion that is produced by γ -secretase (Funamoto *et al*, 2004). Third, swapping tryptophan residues into the γ -site within the APP transmembrane domain prevents γ -cleavage but allows ϵ -cleavage; however, swapping tryptophans into the ϵ -site leads to proteolysis between the γ -site and ϵ -site, at a so-called ζ -site (Fig 1, and see below; Sato *et al*, 2005). Installing tryptophans into the ζ -site prevents any transmembrane cleavage of APP. Therefore, with these tryptophan swaps, ϵ -cleavage can occur without γ -cleavage, but γ -cleavage is not seen without ϵ -cleavage or ζ -cleavage. Interestingly, longer A β peptides resulting from cleavage at the ζ -site are seen intracellularly on treatment with one particular γ -secretase inhibitor: a dipeptide analogue called DAPT (Yagishita *et al*, 2006). Finally, a mutation in PS1—M233T—leads to alternative ϵ -cleavage, producing the 51-residue AICD and its counterpart A β 48 in a cell-free assay with detergent-solubilized membranes (Kakuda *et al*, 2006).

One way to explain two major cleavage sites would be the presence of two pairs of catalytic aspartates within a presenilin dimer at the core of the γ -secretase complex. Several laboratories have reported evidence for presenilin–presenilin interaction (for example, Schroeter *et al*, 2003). However, recent evidence from our laboratory suggests that immunoprecipitation of one epitope-tagged presenilin does not bring down a co-expressed differentially tagged counterpart, but γ -secretase activity is nevertheless

found in the immunoprecipitate (T. Sato & M.S.W., unpublished data). Therefore, two presenilin molecules per complex are not required for proteolytic activity: one presenilin suffices to generate the normal γ -cleaved A β and ϵ -cleaved AICD. Furthermore, cysteine mutagenesis and disulphide crosslinking experiments show that the key aspartate in transmembrane domain 6 is adjacent to the key aspartate in transmembrane domain 7, with no evidence for dimeric presenilin (Tolia *et al*, 2006). Together, these findings support a model of the γ -secretase complex in which one presenilin, and therefore one pair of aspartates, is sufficient to cut the transmembrane domain of APP, and other substrates, in at least two places.

Ihara and co-workers have suggested that these and other observations are consistent with successive cleavage events: initial proteolysis at the ϵ -site leads to the release of AICD, but the long A β products (A β 49 or A β 48) remain in the active site, and are successively cleaved every three residues upstream at the ζ -sites and then again at the γ -sites (Fig 1; Qi-Takahara *et al*, 2005). Specifically, they propose that A β 49 is processed to A β 46, A β 43 and A β 40, whereas A β 48 is trimmed to A β 45, A β 42 and A β 39. This model of successive proteolysis from the ϵ -site to the γ -sites elegantly explains how so many presenilin mutations can both reduce proteolytic activity—causing a loss of function—and increase the A β 42/A β 40 ratio—causing a gain of function. Mutant versions of the enzyme that are less proteolytically efficient also cut proportionately more at the alternative ϵ -site, producing A β 48 (Sato *et al*, 2003). The slower mutant enzymes allow proportionately more release of A β 42 before further trimming to A β 39. The net result might be less total A β , including less A β 40 (Bentahir *et al*, 2006), but the ratio of A β 42 to A β 40 is elevated. In cases in which the proteolytic efficiency of the enzyme is only slightly reduced, the resulting increase in substrate levels might ultimately lead to compensation and little change in total A β ; nevertheless, the reduced efficiency would cause an increase in A β 42/A β 40. This same mechanism might also account for the changes in A β 42/A β 40 seen with Alzheimer-causing APP mutations that are located near the γ -secretase cleavage sites; in these cases, the mutant substrates might be processed less efficiently by the wild-type protease.

The scenario described above, which is supported by numerous reports, also provides an explanation for why the deletion of three out of four presenilin alleles in mice—with only one PS1 allele remaining—does not result in elevated A β 42/A β 40 (Lai *et al*, 2003; although see Refolo *et al*, 1999): the remaining γ -secretase complexes are wild type, have normal proteolytic activity and trim ϵ -cleaved A β efficiently. This is also consistent with the fact that although more than 100 missense mutations in PS1 and PS2 have so far been associated with AD, none are complete loss-of-function mutations in an allele, for example, complete deletion, loss of expression, mutation of one of the catalytic aspartates or severe truncations. Conditional knockout of presenilins in the brain can apparently result in memory impairment and age-dependent neurodegeneration (Saura *et al*, 2004); however, without either A β -containing plaques or tau-containing tangles, this is arguably not a phenocopy of AD. Apparently, presenilin is crucial for neuronal survival and/or replacement in the brain, and complete knockout of both alleles has serious consequences. However, many other genes that are unrelated to AD but are also needed for maintaining neuronal density would be expected to do the same on complete knockout in the adult brain.

As discussed earlier, the presenilin-containing γ -secretase complex processes many other type I integral membrane proteins besides APP. Could reduced or altered proteolysis of one of these other substrates be the true underlying molecular cause of AD, with reduced or altered APP proteolysis being an epiphenomenon? Could the AD-causing mutations in APP be only indirectly pathogenic by tying up more γ -secretase complexes and reducing the proteolysis of another key substrate? This possibility remains real but unlikely, unless AD-causing mutations are found in one of these alternative substrates with concomitant tau pathology but no change in either the level of A β or the ratio of A β 42 to A β 40. So, although reduced and/or altered processing of other substrates is often observed with mutant presenilins—and might exacerbate the AD phenotype in some cases—suggestions that such alterations might be the primary cause of these familial AD cases are speculative. These alterations—for example, reduced Notch signalling—might nevertheless be secondary contributors to neurodegeneration (Beglopoulos & Shen, 2006).

Finally, the emerging role of soluble A β assemblies in synaptic dysfunction and neuronal loss should be mentioned (Selkoe, 2002). A major objection to the amyloid hypothesis—with consequent searches for other reasons why presenilin mutations cause AD—is the lack of correlation between amyloid plaque counts with disease severity or onset. However, a clear correlation is observed under complete solubilization of A β from post-mortem brains using formic acid (Naslund *et al*, 2000). Moreover, soluble A β oligomers, but not monomers, secreted from cells in culture have substantial effects on long-term potentiation after their injection into the brains of living mice. Therefore, even subtle changes in A β levels or in the A β 42/A β 40 ratio might lead to soluble aggregates that are able to cause AD during the course of years or decades.

Some presenilin mutations might cause AD by simply decreasing A β 40 as a net effect on A β production (Bentahir *et al*, 2006). Other evidence also indicates that A β 40 might be protective to the degree that this shorter peptide helps to keep A β 42 from aggregating (T.E. Golde, personal communication). Such evidence should not be taken to mean that γ -secretase inhibition will cause or exacerbate AD: inhibition should reduce A β 42 as well as A β 40, and therefore prevent A β aggregation and downstream toxic effects. Similarly, compounds that selectively decrease A β 42, such as certain non-steroidal anti-inflammatory drugs (Weggen *et al*, 2001), should be beneficial. However, compounds that selectively lower A β 40 would have the potential to promote A β 42 aggregation. No such compounds have been reported so far, and any that are identified in the future should be avoided as therapeutic candidates. Inhibition of the presenilin-containing γ -secretase complex might indeed lead to mechanism-based toxic effects, for example, through reduced Notch signalling; however, the recent discovery of selective γ -secretase modulators that inhibit the processing of APP while allowing Notch processing to continue (Fraering *et al*, 2005; Netzer *et al*, 2003) promises to be an important therapeutic approach. Efforts to identify A β -decreasing agents that work at the level of γ -secretase should be vigorously pursued. Arguments to the contrary, on the basis of a simplistic idea of presenilin loss of function in AD pathogenesis, might impede the discovery and development of life-saving medicines that considerably slow or stop disease progression.

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Hardy J (2007) Putting presenilins centre stage. This issue p134.
De Strooper B (2007) Loss-of-function presenilin mutations in Alzheimer disease. This issue p141.