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Unlocking truths of γ -secretase in Alzheimer's disease: what is the translational potential?

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Abstract

Considerable evidence, particularly from genetics, points to the aggregation-prone amyloid β -peptide as a pathogenic entity in Alzheimer's disease. Hence, the proteases that produce this peptide from its precursor protein have been prime targets for the development of potential therapeutics. One of these proteases, γ -secretase, has been a particular focus. Many inhibitors and modulators of this membrane-embedded protease complex have been identified, with some brought into late-stage clinical trials, where they have spectacularly failed. The reasons for these failures will be discussed, along with recent findings on the mechanism of γ -secretase and of Alzheimer-causing mutations that may suggest new strategies for targeting this enzyme.

Keywords

amyloid; biochemistry; genetics; inhibitors; modulators; protease

Alzheimer's disease (AD) is a progressive and ultimately fatal neurodegenerative disorder and major public health crisis, with over 5 million affected in the USA and some 30 million worldwide. With age being the greatest risk factor and more people expected to live well into old age, AD is projected to dramatically rise in the coming decades [1]. Despite the devastating course of AD, the numbers afflicted, and the huge social and economic costs, current treatments are only symptomatic and marginally effective. No therapeutic agents are available that slow or stop the inexorable neurodegeneration and progressive loss of learning and memory that are well-known hallmarks of the disease. Toward the discovery of such agents, the underlying molecular mechanisms must be understood and in sufficient detail to formulate appropriate strategies for effective prevention or treatment.

The deposition of extracellular amyloid plaques and neurofibrillary tangles in the brain are cardinal pathological features of AD [2]. The former are composed primarily of the amyloid

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β -peptide ($A\beta$), while the latter are comprised of filaments of the otherwise microtubule-associated protein tau. The question of whether either of these two pathological features are involved in the pathogenesis or merely coincidental with the true pathogenic entities has been much debated, but the general consensus is that $A\beta$ and tau are both critical to the neurodegenerative process of AD, with $A\beta$ being upstream of tau in the pathway.

However, the plaques and tangles *per se* may indeed be markers rather than causal agents. In the past decade or so, evidence has mounted suggesting that soluble oligomeric forms of $A\beta$ cause synaptic dysfunction and are more detrimental than the plaques [3], and the same may also be true for tau [4]. Regardless of the exact forms responsible for eliciting synaptic and neuronal toxicity, genetic mutations associated with dominant hereditary dementia clearly point to alterations in $A\beta$ or tau being sufficient for neurodegenerative disease [5,6]. Moreover, $A\beta$ pathology in AD is coupled with tau pathology, while tau-containing neurofibrillary tangles are a common feature of a range of other neurodegenerative diseases [7], suggesting that a variety of neuronal insults, including aberrant $A\beta$, can trigger pathogenic tau.

$A\beta$ basics

$A\beta$ is a 38–43 amino acid secreted peptide derived from the amyloid β -protein precursor (APP), a 695–770 amino acid single-pass transmembrane protein (Figure 1A) [3]. The large extracellular/luminal domain of APP is shed from the membrane by either α -secretase or β -secretase activity. α -secretases are membrane-anchored metalloproteases that cut APP within the $A\beta$ region, thus precluding $A\beta$ production, leaving an APP C-terminal fragment (APP CTF- α) in the membrane. β -secretase cuts further away from the transmembrane domain to generate the N-terminal region of $A\beta$ as part of APP CTF- β . Both APP CTF- α and - β are subsequently cleaved by γ -secretase to produce p3 (from CTF- α) and $A\beta$ (from CTF- β) and the APP intracellular domain (AICD).

The biological roles of APP and its proteolytic fragments are largely unknown [8]. $A\beta$ can interact with nicotinic acetylcholine receptors to modulate its activity [9], although it is unclear if this is a normal and physiologically relevant role. The AICD has been suggested to translocate to the nucleus, where it may interact with certain transcription factors and control gene expression; however, none of the candidate genes have been sufficiently validated [10]. Secreted APP ectodomain has been reported to regulate neuronal apoptosis (pruning) during development [11], although again, this has not been confirmed. A variety of roles have been suggested for full-length APP, including cell proliferation, differentiation, neurite outgrowth, cell adhesion and synaptogenesis [8].

The predominant form of $A\beta$ is composed of 40 amino acids, while ~5–10% is 42 amino acids, with the difference at the C-terminus, which is formed by cleavage of the transmembrane domain of APP by γ -secretase [12]. Although $A\beta_{42}$ is a minor variant, this peptide is much more prone to aggregation than is $A\beta_{40}$ [13] and is the primary component of the amyloid plaques in the AD brain [14]. $A\beta_{42}$ has been extensively studied for many years to understand the aggregation process; the structure of the monomer, oligomers, protofibrils, fibrils and plaques [15]; the neurotoxic species and possible neurotoxic

pathways [16]. The current dogma is that one or more soluble oligomeric forms of A β 42 are the neurotoxic entity, although this leading hypothesis has been met with reasonable skepticism [17].

Genetics

The strongest evidence for the pathogenicity of A β in AD comes from genetics [5]. Dominant missense mutations in the *APP* gene are associated with early-onset familial AD (FAD) with virtually 100% penetrance [18]. Although APP is a large membrane protein, all the FAD mutations in APP are found in and around the small A β region and alter the production or aggregation propensity of A β peptides. An FAD double mutation found immediately proximal to the N-terminus of A β is cleaved more effectively by β -secretase, leading to increased A β production [19,20]. Recently, a protective mutation in this region was discovered, substantially reducing the risk of developing AD, even when carriers reach their 80s or 90s [21]. This protective mutation decreases cleavage by β -secretase, leading to lower production of A β . Other mutations found within the A β sequence increase its aggregation propensity (e.g., [22]). Still others, found near the γ -secretase cleavage site, lead to increased ratios of A β 42 to A β 40 (e.g., [23]), thereby increasing the aggregation propensity as well.

Dominant FAD missense mutations are also found in genes encoding presenilin-1 and -2 (PS1 and PS2), multipass membrane proteins that comprise the catalytic component of γ -secretase [5]. Over 100 such mutations have been identified [18], and some can cause AD even before age 30 [24]. Like those FAD mutations in APP near the γ -secretase cleavage site, FAD-mutant PS1 and PS2 lead to increased A β 42/A β 40 and overall increased aggregation propensity [25–28]. Other than the strong heredity factor and early age of onset, these dominant missense mutations in APP and the presenilins result in a similar course of disease onset, progression and brain pathology (including neurofibrillary tangles containing tau filaments) that are observed in sporadic, late-onset AD.

While dominant mutations in the gene encoding tau can also cause neurodegenerative diseases, AD is not one of them; rather, these mutations typically result in frontotemporal lobar degeneration (FTLD) [7,29]. FTLD-associated tau mutations, however, lead to similar tau-based neuropathology (intraneuronal filaments and neurofibrillary tangles) that are seen in AD. FTLD-associated mutations in the tau gene (*MAPT*) are of two types: missense mutations and silent or intronic mutations. The former mutations change the sequence of the protein and can increase its propensity to aggregation [30–37], while the latter mutations change alternative RNA splicing to increase the proportion of a particular tau isoform [38,39]. Like A β , it is unclear which form of tau is pathogenic. Nevertheless, the discovery of these mutations demonstrated that changes in tau alone are sufficient for tau pathology, neurodegeneration and dementia. Mutation of *MAPT* is apparently one of several ways to elicit tau pathogenicity, with aberrant A β being the trigger in AD [40].

As for sporadic late-onset AD, the strongest genetic risk factor is apolipoprotein E (*APOE*) genotype [41]. Apolipoprotein E is a cholesterol-carrying protein with three allelic variants (E2, E3 and E4). One copy of the E4 allele increases the risk of late-life AD by threefold to

fourfold; two copies increases the risk 12- to 15-fold. The ApoE4 allele is responsible for many more cases of AD than the FAD mutations found in APP and the presenilins: FAD is quite rare, while sporadic, late-onset AD is much more common. How ApoE4 increases the risk of AD is not clear, although evidence supports decreased A β clearance from the brain [42]. While the FAD cases are very rare, because the presentation, course and pathology are similar to sporadic AD, the mechanisms by which the FAD mutations result in neurodegeneration and dementia should be informative about the pathogenic mechanism of AD in general. For this reason, A β and A β production, particularly via proteolytic cleavage by γ -secretase, has been such a major focus in the field. FAD mutations alter A β or A β production, with the large majority of these mutations found in the presenilins, which alter γ -secretase activity to increase A β propensity to aggregation. In the next section, we discuss the details of γ -secretase biochemistry and the effects of FAD mutations on its activity.

Biochemistry

As mentioned earlier, presenilin is the catalytic component of γ -secretase. Specifically, two completely conserved transmembrane aspartic acids are required for proteolytic activity [43], placing γ -secretase in the aspartyl protease family, even though it otherwise bears no resemblance to the many well-known soluble aspartyl proteases (e.g., pepsin, renin, HIV protease). The presence of the two transmembrane aspartates within a multipass membrane protein is consistent with the ability of γ -secretase to cleave within the transmembrane domain of APP in generating A β . Three other membrane proteins are components of γ -secretase along with presenilin: nicastrin, Aph-1 and Pen-2 [44–46]. After all four components assemble in the endoplasmic reticulum, presenilin undergoes autoproteolysis [43,47] into an N-terminal fragment (NTF) and C-terminal fragment (CTF) to generate the active γ -secretase complex (Figure 1B). Each of these presenilin subunits contributes one of the catalytic aspartates to the active site; that is, the active site is located at the interface between presenilin NTF and CTF [48,49]. Although several other proteins have been reported as activators or modulators of γ -secretase [50–52], none of these have been validated. The protease is trafficked through the secretory pathway to the cell surface [53–55]. APP proteolysis takes place everywhere from the Golgi apparatus to the cell membrane and endocytic vesicles [56–59].

γ -secretase has many more substrates besides APP [60]. Indeed, so many substrates have been identified that the γ -secretase complex has been called the proteasome of the membrane [61], implying that one of its major functions is to clear out membrane protein stubs that remain after ectodomain release by sheddases. While membrane protein clearance may be an important function of γ -secretase, the protease also plays essential roles in certain cell signaling pathways. The most important of these is signaling from the Notch family of receptors [62]. Notch receptors are single-pass membrane proteins such as APP, and proteolytic processing of Notch, triggered by interaction with cognate ligands on neighboring cells, leads to release of its intracellular domain. The Notch intracellular domain translocates to the nucleus and interacts with specific transcription factors that control the expression of genes involved in cell differentiation. These signaling pathways, particularly from Notch1 receptors, are essential to proper development in all multicellular animals. Knockout of presenilin genes in mice leads to phenotypes that are virtually

identical with those observed upon knockout of the *Notch1* gene [63,64], findings that, as explained in the next section, have major implications for the potential of γ -secretase inhibitors (GSIs) as AD therapeutics.

Inhibitors

The search for GSIs has gone on for over two decades, ever since A β was discovered to be a normally secreted peptide produced from a variety of cell types in culture [65]. Such inhibitors were identified even before the components of the protease were known and ultimately served as critical tools for discovery of presenilin as the catalytic component [48,49]. Initially, these inhibitors were simple peptidomimetics, but pharmaceutical companies quickly developed compounds with much better drug-like properties that allowed *in vivo* testing for the ability to lower A β in the brains of transgenic AD mice (e.g., expressing FAD mutant APP and presenilins).

Acute treatment with GSIs did show such proof of principle for these drug candidates [66,67]; however, chronic treatment revealed serious peripheral toxicities [68,69], such as gastrointestinal bleeding and immunosuppression and skin lesions, all effects that could be traced to inhibition of Notch1 proteolysis and signaling. As AD patients would be required to take GSIs for years and perhaps decades, the severe toxic consequences of γ -secretase inhibition caused great concern. While there were hopes for a therapeutic window that would allow lowering brain A β levels without the peripheral Notch-deficient toxicity, the failure of one GSI, semagacestat, in Phase III clinical trials, dashed these hopes [70]. The trial resulted in unacceptable peripheral toxicities, and more worrisome, but cognition that was worse than the placebo control groups.

Because all the serious toxic effects were apparently caused by inhibition of Notch1 signaling, the focus then went into finding GSIs that could selectively inhibit the proteolysis of APP by γ -secretase without affecting Notch1 proteolysis. The finding that the γ -secretase complex contained a nucleotide binding site that could selectively influence the cleavage of APP over Notch1 [71,72] led to the discovery of so-called 'Notch-sparing' GSIs [73,74]. The name is somewhat misleading, however, as these compounds show APP/Notch selectivity, not complete lack of effect on Notch proteolysis. Moreover, the degree of selectivity has been a matter of debate, with some reports of a lack of any selectivity for APP [75,76]. One such compound, avagacestat, went as far as Phase II clinical trials, and like semagacestat caused Notch-deficient toxicities at higher doses, equivocal CSF A β lowering at lower doses and worsening of cognition [77]. Evidence from mouse models suggest that the cognitive worsening may be due to increased γ -secretase substrates (APP CTF- α and CTF- β) [78], although elevation of total A β , seen in plasma at low inhibitor concentrations, may be responsible [79,80]. These findings have had a tremendous chilling effect on further development of GSIs for AD, and it is unlikely that another GSI will be brought into clinical trials in the foreseeable future. Interestingly though, these compounds may be repurposed for oncology, as many clinical trials are ongoing for the treatment of various cancers that involve overactive Notch signaling.

Modulators

While GSIs appear to be out of further consideration for AD therapeutics, γ -secretase modulators (GSMs) are still of keen interest. These compounds have the remarkable effect of lowering A β 42 levels without decreasing overall A β levels or otherwise inhibiting general γ -secretase activity [81,82]. The decrease in A β 42 is correlated with an increase in A β 38, thus replacing a highly aggregation-prone form of A β with a much more soluble form. Thus, these compounds can prevent the formation of A β 42-containing oligomers and plaques in the brain. GSMs, however, have no effect, even at very high concentrations, on Notch proteolysis and signaling, nor do they elevate γ -secretase substrates APP CTF- α and CTF- β . Presumably for these reasons, these compounds have shown excellent safety profiles, both in animal models and in human trials.

The mechanism of action of these compounds is not entirely clear, although the correlation between A β 42 lowering and A β 38 elevation is apparently relevant. Strong evidence supports A β 42 as a precursor to A β 38, with γ -secretase cleaving the A β 42 C-terminus to release a tetrapeptide [83]. Although reports of dissociation between A β 42 and A β 38 have been interpreted as evidence against a precursor-product relationship [84,85], the recent finding that isolated γ -secretase converts synthetic A β 42 to A β 38 with release of the tetrapeptide [86] unambiguously confirms the relationship. Moreover, presenilin mutations decrease the A β 42-to-A β 38 conversion while GSMs stimulate it. Thus, GSMs appear to decrease A β 42 by enhancing the carboxypeptidase activity of γ -secretase that converts this aggregation-prone peptide to A β 38.

A critical issue with GSMs, however, like all anti-A β therapeutic strategies, is the design of clinical trials [87]. So far, all reported clinical trials with candidate AD therapeutic agents, including GSIs, GSMs and anti-A β immunotherapy, have been with individuals who already have AD. Even in those with mild or moderate AD, substantial neurodegeneration has occurred, and there are serious concerns that targeting A β after the onset of symptoms is too late. A β pathology in the brain may appear more than 10 years before the clinical manifestation of AD. As A β pathology apparently precedes tau pathology [88], and tau pathology may then propagate from neuron to neuron [89,90], blocking A β after tau pathology is initiated may not prevent or slow the progression of AD and may not even prevent or delay disease onset. For anti-A β strategies, including GSMs, to succeed, clearer knowledge of the pathogenic process and timing is needed, as are convenient and reliable biomarkers and diagnostics.

Moreover, the clinical success of GSMs is completely dependent on whether A β 42 is indeed the pathogenic entity in AD. The reasons for the focus on A β 42 are arguably more historic than a result of an objective search starting with no preconceptions. Over 100 years ago, Alois Alzheimer described extraneuronal amyloid plaques as a signature pathological characteristic of the disease, and the discovery in the late 1980s and early 1990s that the primary protein component of the plaques is A β [91,92] with A β 42 being the predominant species [14] and most aggregation prone [13], led to the assumption that A β 42 is the likely pathogenic species. Subsequent studies ranging from effects of APP and presenilin mutations to the neurotoxicity of various A β assemblies would seem to confirm this

hypothesis. However, selectivity in the reporting of findings (negative results are more difficult to publish) in combination with incomplete knowledge of all forms of A β could result in mistaking correlation for causality. This issue will be addressed in further detail in the next sections.

Processive proteolysis

In recent years, the proteolysis of APP substrates by γ -secretase has been revealed to be considerably more complicated than initially believed. As noted earlier, secreted forms of A β range from 38 to 43 amino acids. However, identification of the N-terminus of the other product of γ -secretase cleavage of APP, AICD, revealed that proteolysis also occurs between residues 49 and 50 (A β numbering) [93], close to the cytosolic end of the transmembrane domain, and this cleavage event is also γ -secretase-dependent. Mass spectrometric analysis of AICD showed that a minor degree of cleavage also occurs between residues 48 and 49 [94]. Interestingly, FAD mutations were found to increase the proportion of AICD beginning at residue 49 over that beginning at residue 50, similar to the ability of these mutations to increase A β 42 over A β 40 [94]. Thus, γ -secretase cleaves the APP transmembrane domain at least twice, and both of these cleavage events are altered by FAD mutations.

In the analysis of γ -secretase cleavage products, however, some residues were unaccounted for. Most A β peptides are A β 40, while most AICD begins at residue 50. The discovery of longer forms of A β was an important step in solving this mystery. Modified gel electrophoresis methods allowed separation of A β peptides to one amino acid resolution, and such analysis revealed A β 45, A β 46, A β 48 and A β 49 [95–97]. Thus, A β peptides ranging all the way to where AICD begins suggested that initial proteolysis occurs near the C-terminal end of the transmembrane domain, producing AICD and either A β 48 or A β 49. These long A β peptides are then trimmed through a carboxypeptidase function of γ -secretase to produce the secreted forms of A β [96,98]. Further studies refined this idea by suggesting two pathways for A β production: A β 49–A β 46–A β 43–A β 40 and A β 48–A β 45–A β 42–A β 38 (Figure 1B) [99]. The expected tri- and tetra-peptides formed in this process have been detected by mass spectrometry in cell-free γ -secretase assays [83]. Interestingly, the small levels of long A β peptides released by the protease complex before complete trimming are found in cell membrane fractions [95], as might be expected given that these peptides contain most of the APP transmembrane domain. A new study shows differences in the proportions of A β products formed by different isoforms of γ -secretase, with Aph-1B and PS2 associated with skewing toward longer A β peptides [100]. Such a finding is consistent with antisense against PS1 expression leading to increased A β 42/A β 40 [101].

Reports of dissociation between A β 42 and A β 38 have led to doubts about the precursor–product relationship between these two peptides [84,85], and other reports of dissociation between A β and AICD have questioned the carboxypeptidase activity of γ -secretase and the dual-pathway model (e.g., [52]). However, as mentioned above, unambiguous evidence that γ -secretase converts A β 42 to A β 38 has recently been provided using *in vitro* assays [86]. Furthermore, a dissociation of A β and AICD at the γ -secretase level is a stoichiometric impossibility, as cleavage of APP CTF- β must give one A β molecule for every AICD, and

this has been formally proven to be the case through careful quantification [96]. Moreover, in unpublished work, we have found that synthetic peptides ranging from A β 45 to A β 49 are converted to A β 40 and A β 42 by purified γ -secretase and in the same proportions as expected from the dual-pathway model [Fernandez MA, Wolfe MS, Unpublished Data]. These results were seen whether using detergent solubilized γ -secretase preparations or purified protease complex reconstituted into lipid vesicles. Thus, the carboxypeptidase activity of γ -secretase and the dual-pathway model are unambiguously established as intrinsic properties of the enzyme independent of the membrane.

Presenilin mutations: new findings

Recent findings on the more detailed effects of presenilin FAD mutations on A β production suggest how these mutations may cause disease. In a study from our laboratory [102], five different FAD PS1-mutant γ -secretase complexes were compared with wild-type using *in vitro* assays and PAGE analysis of the range of A β peptides as well as AICD. Four of the five FAD mutations caused a reduction in AICD and total A β production compared with the wild-type enzyme, consistent with previous findings that the majority of PS1 mutations cause a 'loss of function' in proteolysis. One of these mutations, however, produced AICD and A β to the same degree as the wild-type enzyme, demonstrating that such general reduction of proteolytic function is not essential for pathogenicity. However, all five mutations skewed A β production in favor of the longer forms (>A β 42), suggesting that the mutations all caused a deficiency in the carboxypeptidase function of γ -secretase. Another report from the laboratory of Bart De Strooper [76] confirmed these findings, that presenilin FAD mutations do not necessarily inhibit general proteolysis by γ -secretase but do cause qualitative changes in the types of A β peptides produced, in favor of longer forms. Most recently, in unpublished work we have further found that PS1 FAD-mutant γ -secretase complexes are dramatically deficient in their ability to trim A β 48 and A β 49 to A β 40 and A β 42, providing important confirmation of reduced carboxypeptidase function as the common feature of disease-causing γ -secretase, independent of initial cleavage of APP CTF- β to form AICD and A β 48/49 [Fernandez MA, Wolfe MS, Unpublished Data].

Collectively, these recent findings show that the increase in A β 42-to-A β 40 observed by presenilin FAD mutations may be a consequence of reduced trimming function. However, the relationship between these two A β peptides is not the only change. In general, the proportion of longer forms of A β , including membrane-associated forms A β 45-A β 49, are substantially increased, begging the question of what pathogenic role, if any, these A β peptides may play. In any event, the apparent reduction in carboxypeptidase function caused by presenilin FAD mutations suggests that therapeutic agents targeting γ -secretase should correct this biochemical defect; that is, rather than searching for inhibitors of γ -secretase, stimulators of the carboxypeptidase function should be sought. The GSMs identified to date do this, but only for the last of the trimming step (e.g., A β 42 to A β 38), making their therapeutic potential entirely dependent on A β 42 being the primary pathogenic species. A more general stimulator of γ -secretase carboxypeptidase activity might have a better chance of working, as it would not depend on knowing exactly which form of A β is responsible for the cascade of events that result in neurotoxicity, neurodegeneration and dementia.

Conclusion

Presenilin and the γ -secretase complex are central to the pathogenesis of AD, as dominant mutations in the presenilins alter γ -secretase activity and A β production and cause hereditary AD in midlife that is otherwise indistinguishable from sporadic, late-life AD. The search for potential AD therapeutics that target γ -secretase has gone on for many years, even before the discovery of the components of this membrane-embedded protease complex and the plethora of other substrates besides APP. Potent brain-penetrant inhibitors have been identified and brought into clinical trials; however, these compounds show serious toxic effects due to inhibition of proteolysis and signaling from the Notch1 receptor. More worrisome, cognitive worsening was observed, which may be due to increased levels of APP γ -secretase substrates. Modulators of γ -secretase have been discovered that selectively lower A β 42 and are currently in clinical trials. Although these compounds are apparently safe, their potential to prevent or treat AD is dependent on A β 42 being the primary pathogenic entity. New findings on the effects of AD-causing presenilin mutations reveal a common defect in carboxypeptidase function, resulting in increased proportions of other A β peptides, even longer than A β 42. The pathogenicity of these longer A β peptides is unknown.

Future perspective

Given the clear failure of GSIs in the clinic for the treatment of AD, such agents will likely not be further pursued any time in the foreseeable future, although they may have potential for the treatment of cancers involving overstimulated Notch signaling [103]. GSMs remain viable candidates, but clinical evidence that these agents slow or stop AD progression is so far lacking. Independent evidence that A β 42 is indeed a worthwhile target (e.g., via immunotherapy) would increase interest in GSMs for AD. New evidence shows that presenilin mutations reduce the carboxypeptidase function of γ -secretase to increase proportions of longer forms of A β , suggesting that more general stimulators of this trimming function might be more appropriate. However, all anti-A β strategies would be expected to fail as treatments for AD patients if pathogenic A β triggers neurotoxic events that then become A β -independent (e.g., propagation of pathological tau from neuron to neuron). The potential of these agents for prevention will require identification of appropriate biomarkers that enable the formulation of convenient diagnostic tests capable of discerning those pre-symptomatic individuals who are at high risk of developing AD.

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EXECUTIVE SUMMARY

Background

- Current treatments for Alzheimer's disease (AD) are symptomatic and largely ineffective.
- Evidence strongly supports the amyloid β -peptide ($A\beta$) as the pathogenic initiator of AD. In particular, dominant mutations in genes encoding the $A\beta$ precursor protein (APP) and the presenilins all alter $A\beta$ properties or production.
- Presenilin is the catalytic component of γ -secretase, a protease that determines the length of $A\beta$ peptides.

Current approaches

- Inhibitors of γ -secretase have failed in clinical trials, causing toxicities resulting from blocking the proteolysis of another substrate and cognitive worsening that may be due to elevation of APP substrates.
- Modulators of γ -secretase can selectively lower $A\beta_{42}$ and appear to be safe, but their potential as AD therapeutics is unclear.
- Presenilin mutations specifically reduce the trimming function of γ -secretase, which converts long $A\beta$ peptides to shorter secreted forms.

Future prospects

- Stimulation of the trimming function of γ -secretase would take an unbiased view of which particular $A\beta$ peptide is pathogenic.
- All anti- $A\beta$ strategies may require diagnostics that identify at-risk individuals years before the onset of symptoms.

Figure 1. Production of amyloid β -peptide from amyloid precursor protein

(A) APP is an integral membrane protein, with the $A\beta$ portion derived in part from the single transmembrane domain. β -secretase (β scissors) cleaves outside the membrane to release the large extracellular domain of APP. The membrane-bound remnant is then cleaved within the transmembrane domain by γ -secretase (γ -scissors) to produce $A\beta$ and the AICD. The aggregation-prone $A\beta$ peptide forms neurotoxic oligomers and amyloid plaques. (B) γ -secretase is composed of four different membrane proteins, with the presenilin N-terminal and C-terminal fragment heterodimer as the catalytic component that carries out proteolysis within the APP transmembrane domain. The protease complex initially cuts at the ϵ sites to produce long $A\beta$ peptides A β 48 or A β 49. The enzyme then trims these long $A\beta$ peptides in increments of 3–4 amino acids (at the ζ , γ and γ' sites) along the A β 40 or A β 42 pathways. $A\beta$: Amyloid β -peptide; AICD: Amyloid precursor protein intracellular domain; APP: Amyloid precursor protein.