



Dysfunctional γ -Secretase in Familial Alzheimer's Disease

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Abstract

Genetics strongly implicate the amyloid β -peptide ($A\beta$) in the pathogenesis of Alzheimer's disease. Dominant missense mutation in the presenilins and the amyloid precursor protein (APP) cause early-onset familial Alzheimer's disease (FAD). As presenilin is the catalytic component of the γ -secretase protease complex that produces $A\beta$ from APP, mutation of the enzyme or substrate that produce $A\beta$ leads to FAD. However, the mechanism by which presenilin mutations cause FAD has been controversial, with gain of function and loss of function offered as binary choices. This overview will instead present the case that presenilins are dysfunctional in FAD. γ -Secretase is a multi-functional enzyme that proteolyzes the APP transmembrane domain in a complex and processive manner. Reduction in a specific function—the carboxypeptidase trimming of initially formed long $A\beta$ peptides containing most of the transmembrane domain to shorter secreted forms—is an emerging common feature of FAD-mutant γ -secretase complexes.

Keywords Amyloid · Protease · Genetics · Biochemistry

Introduction

The deposition of extracellular amyloid plaques and neurofibrillary tangles in the brain are cardinal pathological features of Alzheimer's disease (AD) [17]. The former are composed primarily of the amyloid β -peptide ($A\beta$), while the latter are comprised of filaments of the otherwise microtubule-associated protein tau. 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 deposits per se may not be pathogenic. Indeed, amyloid plaques numbers or location do not seem to correlate with disease onset or progression [26, 29, 34]. In the past two decades, evidence has mounted suggesting that soluble oligomeric forms of $A\beta$ cause synaptic dysfunction and are more detrimental than the plaques [28]. Nevertheless, the specific pathogenic form of $A\beta$ remains unknown, and the molecular means by which pathogenic $A\beta$ triggers downstream synapto- and neurotoxic events are unclear [1]. Regardless of the exact forms responsible for eliciting synaptic and neuronal toxicity, genetic mutations associated

with autosomal-dominant familial AD (FAD) clearly point to alterations in $A\beta$ being sufficient for neurodegenerative disease [45].

APP Processing by Secretases

$A\beta$ 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 [28] (Fig. 1). 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 [33].

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 from the transmembrane domain of APP by γ -secretase [10]. Although $A\beta_{42}$ is a

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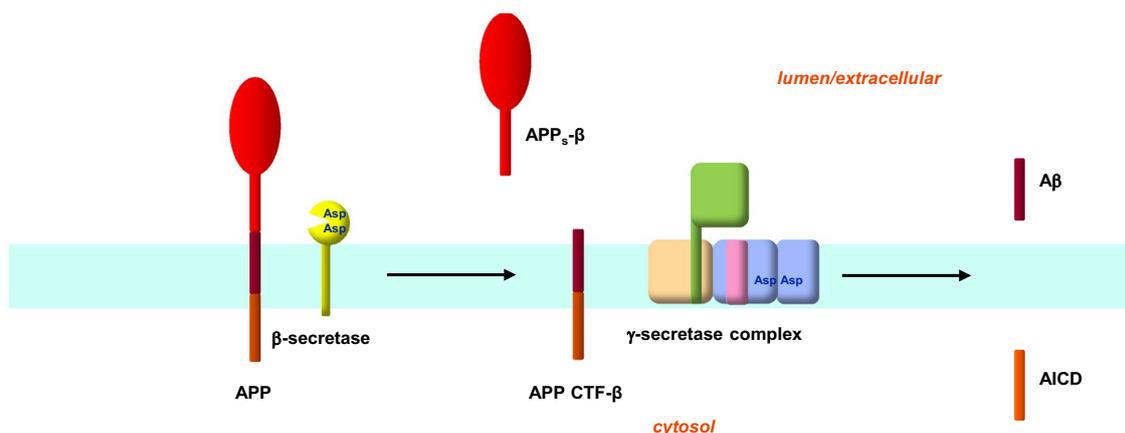


Fig. 1 Production of A β from APP. APP is an integral membrane protein, with the A β portion derived in part from the single transmembrane domain. β -Secretase is a membrane-tethered aspartyl protease in the pepsin family that cleaves APP outside the membrane to release the large extracellular domain of APP (APP_s- β). The membrane-bound remnant APP (CTF- β) is then cleaved by γ -secretase to

produce A β and the APP intracellular domain (AICD). γ -Secretase a membrane-embedded aspartyl protease composed of four different membrane proteins. Presenilin NTF/CTF heterodimer (each subunit containing one transmembrane aspartate) is the catalytic component of the γ -secretase complex that carries out proteolysis within the APP transmembrane domain

minor variant, this peptide is much more prone to aggregation than is A β 40 [21] and is the primary component of the amyloid plaques in the AD brain [20]. A β 42 has been extensively studied for many years to understand the aggregation process; the structure of the monomer, oligomers, protofibrils, fibrils and plaques [39]; and the neurotoxic species and possible neurotoxic pathways [32]. 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 [1].

Genetics of Familial AD

The strongest evidence for the pathogenicity of A β in AD comes from genetic studies of early-onset familial AD (FAD) [45]. Dominant missense mutations in the APP gene are associated with FAD. 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 [4, 6]. Recently, a protective mutation in this region was discovered, substantially reducing the risk of developing AD, even when carriers reach their 80s or 90s [22]. 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., 27]. Still others, found near the γ -secretase cleavage site, lead to increased ratios of A β 42 to A β 40 [e.g., 42], thereby increasing the aggregation propensity as well.

Dominant FAD missense mutations are also found in genes encoding presenilin-1 and -2 (PS1 and PS2), multi-pass membrane proteins that comprise the catalytic component of γ -secretase [45]. Over 200 such mutations have been identified, and some can cause AD even before age 30 [31]. 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 [2, 7, 46, 49]. 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 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.

The γ -Secretase Complex

As mentioned above, presenilin is the catalytic component of γ -secretase. Specifically, two completely conserved transmembrane aspartic acids are required for proteolytic

activity [48], 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 multi-pass 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 [12, 24, 44]. After all four components assemble in the endoplasmic reticulum, presenilin undergoes autoproteolysis [15, 48] into an N-terminal fragment (NTF) and C-terminal fragment (CTF) to generate the active γ -secretase complex (Fig. 1). Each of these presenilin subunits contributes one of the catalytic aspartates to the active site [13, 25].

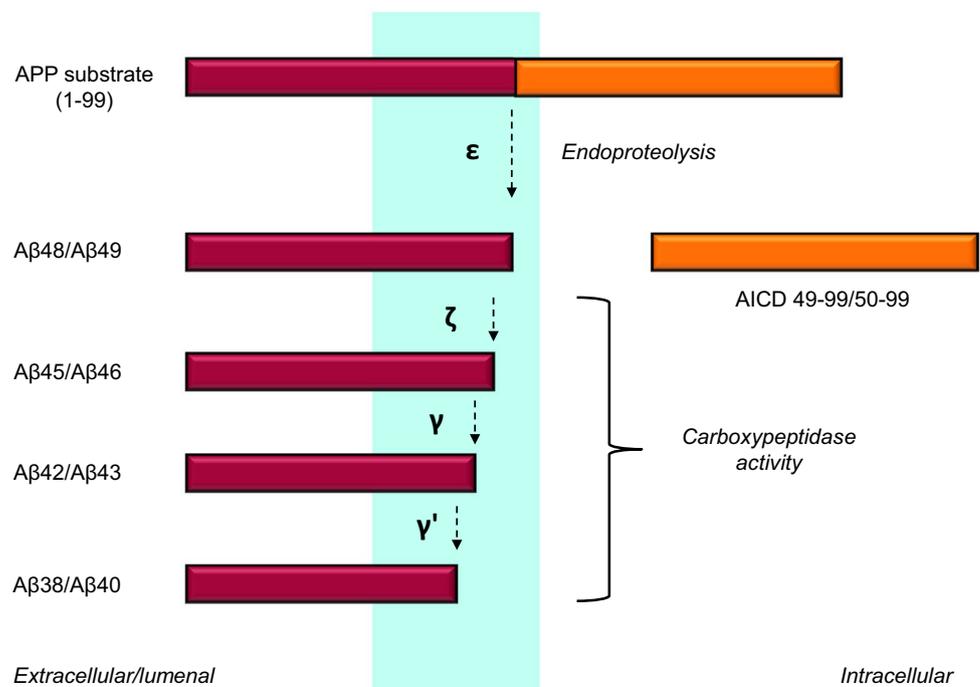
γ -Secretase has many more substrates besides APP [18]. The most important of these is signaling from the Notch family of receptors [41]. Notch receptors are single-pass membrane proteins like APP, and proteolytic processing of Notch, triggered by interaction with cognate ligands on neighboring cells, leads to release of its intracellular domain. This released domain then 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 multi-cellular animals.

Processive Proteolysis by γ -Secretase

In recent years, the proteolysis of APP substrates by γ -secretase has been revealed to be much 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) [47], 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 [40] (Fig. 2). 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 [40]. 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 resolution of A β peptides to one amino acid resolutions, and such analysis revealed A β 45, A β 46, A β 48 and A β 49 [23, 37]. 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

Fig. 2 Processive proteolysis of the APP transmembrane domain by γ -secretase. An endoproteolytic activity of the enzyme cleaves at the ϵ site within the transmembrane domain close to the membrane–cytosol interface to give long A β peptides A β 48 and A β 49 and the APP intracellular domain (AICD). The carboxypeptidase activity of γ -secretase then trims A β 48 and A β 49 in 3–4 amino acid increments



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 [16, 23] (Fig. 2) Further studies refined this idea by suggesting two pathways for A β production: A β 49 \rightarrow A β 46 \rightarrow A β 43 \rightarrow A β 40 and A β 48 \rightarrow A β 45 \rightarrow A β 42 \rightarrow A β 38 [50] (Fig. 3). The expected tri- and tetrapeptides formed in this process have been detected by mass spectrometry in cell-free γ -secretase assays [43]. Interestingly, the small levels of long A β peptides released by the protease complex before complete trimming are found in cell membrane fractions [37], as might be expected given that these peptides contain most of the APP transmembrane domain.

Reports of dissociation between A β 42 and A β 38 led to doubts about the precursor-product relationship between these two peptides [9, 36], and other reports of dissociation between A β and AICD have questioned the carboxypeptidase activity of γ -secretase and the dual-pathway model

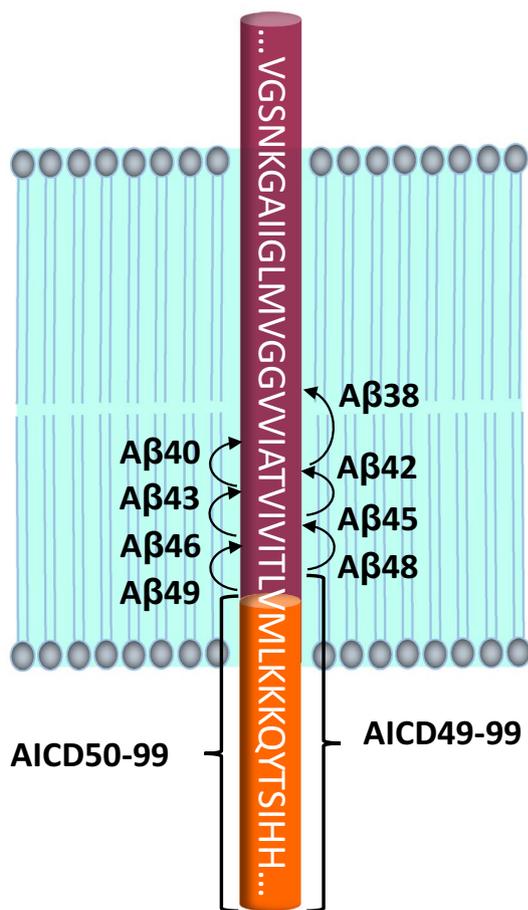


Fig. 3 Dual-pathway proteolysis of APP by γ -secretase. Cleavage at the ϵ site occurs at one of 2 sites, resulting in A β 49 and AICD50-99 or A β 48 and AICD49-99. Subsequent carboxypeptidase trimming leads to two A β product lines: A β 49 \rightarrow A β 46 \rightarrow A β 43 \rightarrow A β 40 and A β 48 \rightarrow A β 45 \rightarrow A β 42 \rightarrow A β 38

[e.g., 19]. However, as mentioned above, unambiguous evidence that γ -secretase converts A β 42 to A β 38 has come from in vitro assays [35]. Furthermore, a dissociation of A β and AICD 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 [23]. Moreover, we have found that synthetic peptides ranging from A β 45–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 [14]. 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.

FAD γ -Secretases and Carboxypeptidase Activity

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 lab [38], five different FAD PS1-mutant γ -secretase complexes were compared to 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 to 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 lab of De Strooper [5] 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. 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 [14], 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.

Collectively, these 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, raising 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. A class of γ -secretase modulators do this, but only for the last of the trimming step (e.g., A β 42 \rightarrow 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 be more appropriate, 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.

Pathogenic and Therapeutic Implications

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. This membrane-embedded protease has been considered an AD therapeutic target for AD for over 20 years. Potent brain-penetrant inhibitors have been developed and brought into clinical trials. However, these compounds showed serious toxic effects due to inhibition of proteolysis and signaling from the Notch1 receptor [8, 11]. More worrisome, cognitive worsening was observed, which mouse models suggest may be due to increased levels of APP γ -secretase substrates [30]. Modulators of γ -secretase have been discovered that selectively lower A β 42 and some have entered clinical trials [3]. 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.

While possible roles of these long A β peptides in AD should be investigated, parallel efforts to identify compounds that rescue the deficient carboxypeptidase function of FAD-mutant γ -secretase complexes may lead to the development of effective therapeutics for the prevention of FAD. Regardless of the pathogenic form of A β , rescuing deficient carboxypeptidase function so the mutant enzyme works more like the wild-type enzyme should be beneficial to those carrying these mutations. Presenilin mutation carriers are fated to develop this devastating form of the disease

in mid-life. While only 1–2% of all AD cases are FAD, this still represents many tens of thousands of individuals who otherwise currently have no hope of escaping this fate. The discovery of the specific biochemical defect of FAD-mutant γ -secretase paves the way for drug discovery efforts specifically for FAD.

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