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Review

Amyloid precursor protein carboxy-terminal fragments as catalyzers of endolysosomal dysfunction in Alzheimer's disease

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Proteolytic processing of the amyloid precursor protein (APP) generates not only the well-known β-amyloid (Aβ) peptides but also APP C-terminal fragments (APP-CTFs). Recent evidence from studies in murine- or human-derived (neuronal) models suggests that APP-CTFs may independently contribute to Alzheimer's disease (AD) pathology by disrupting cellular homeostasis. This review highlights pathological effects unique to APP-CTFs that are independent of Aβ, shedding light on their distinct role in disease progression. We explore the mechanisms underlying APP-CTF-induced toxicity, with a focus on their contribution to endolysosomal dysfunction. APP-CTFs impair lysosomal function and disrupt calcium signaling between the endoplasmic reticulum and lysosomes, compounding organelle dysfunction. Understanding these mechanisms will aid the design of preventive therapeutic strategies that take into account the impact of APP-CTFs on AD pathology.

Highlights

Emerging evidence identifies amyloid precursor protein C-terminal fragments (APP-CTFs) as critical mediators of neuronal dysfunction, independently from β-amyloid peptides.

γ-secretase-mediated intramembrane proteolysis may be required to abrogate APP function. In the context of Alzheimer's disease (AD), APP-CTFs may operate as a primary trigger by disrupting endolysosomal homeostasis.

Mechanistically, APP-CTFs accumulation at late endosomes/lysosomes–endoplasmic reticulum contacts impairs ion and lipid signaling.

APP-CTF toxicity is underappreciated in advancing preventive therapeutic strategies in AD.

Tackling AD: the case for early action

AD, the most common neurodegenerative disorder, affects over 55 million people worldwide. It is marked by two main brain lesions: (i) extracellular amyloid plaques composed of Aβ, and (ii) intraneuronal fibrillary tangles containing hyper-phosphorylated tau. Over the past decades, diagnosis has advanced through tools such as the Mini-Mental State Examination (MMSE) for cognitive impairment, Aβ and tau biomarkers in cerebrospinal fluid or plasma, and magnetic resonance imaging (MRI) for cerebral atrophy. Positron emission tomography (PET) with ^{18}F-fluorodeoxyglucose can identify metabolic dysfunction whereas specific PET ligands can detect Aβ, tau, or activated microglia. Despite these diagnostic advancements, AD remains incurable. Until recently, only symptomatic treatments were available: (i) cholinesterase inhibitors to compensate for cholinergic neuron loss in AD, and (ii) N-methyl-D-aspartate (NMDA) receptor antagonists to mitigate excitotoxicity [1].

Since the discovery of Aβ and tau accumulation in AD in the mid-1980s, which formed the basis of the 'amyloid cascade hypothesis' introduced in the early 1990s, much research has focused on limiting amyloid accumulation and enhancing its clearance from the brain. Indeed, according to this hypothesis, excess Aβ – from aberrant APP metabolism and/or impaired Aβ clearance (e.g., by microglia) – promotes its aggregation and deposition, which initiates metabolic stress and subsequent pathogenic events including neurofibrillary tangles, inflammation, synaptic dysfunction, neuronal death and dementia [2]. On this basis, many Aβ clearing immunotherapies have been developed. Recently, the FDA-approved Leqembi® (Lecanemab) demonstrated a delay of approximately 5 months in cognitive decline during a Phase 3 clinical trial [3]. Additionally, Trontinemab® (formerly Gantenerumab), currently in a Phase 1/2 trial, effectively cleared amyloid plaques in a dose-dependent manner [4].

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However, most of these treatments primarily target amyloid already present in the brain. Aside from ongoing clinical trials such as the DIAN-TU primary prevention trial aiming to treat high-risk individuals before the advent of clear amyloid deposition [5], such strategies offer limited solutions for prevention. On the other hand, pharmacological inhibitors of β- or γ-secretases, the enzymes releasing Aβ from APP, have been associated with severe adverse effects [6–10]. This highlights a critical gap in our understanding of the biology of these proteases.

As mentioned, most therapeutic strategies focused on alleviating excess (toxic) Aβ peptides; with that, their direct precursors – APP-CTFs – have also been long recognized as potentially toxic, yet remained relatively underexplored. Indeed, the precursor–product relationship between APP-CTFs and Aβ complicates disentangling their individual toxicities, as changes in one inevitably influence the other. Recent studies in murine and human neuronal models have highlighted the role of APP-CTFs in AD pathogenesis, particularly their ability to disrupt endolysosomal homeostasis [11–14]. This disruption is a prominent preclinical indicator of neuronal demise in AD and related dementias [15,16]. In this review, we discuss emerging evidence from *in vivo* mouse models, post-mortem human brain tissue and *in vitro* mouse and human neuronal models that sheds light on the molecular mechanisms underlying the cellular toxicity of APP-CTFs, particularly in relation to their impact on endosomal and lysosomal homeostasis. Previous reviews have thoroughly explored the generation, aggregation, and toxicity of Aβ species (e.g., [17,18]), but the role of APP-CTFs, especially in organellar dysfunction, has remained comparatively underappreciated. We focus here on a critical survey of this emerging facet of APP biology, providing mechanistic insights and identifying gaps in knowledge. Finally, we discuss the potential therapeutic implications of targeting APP-CTFs and related proteolytic intermediates as complementary or alternative strategies to current amyloid-focused approaches.

Focusing on APP proteolysis: APP-CTFs at the crossroads

APP-CTFs are primarily generated through ectodomain shedding of APP by α- or β-secretase, producing CTFs of different lengths, termed APP-αCTF (or C83) and APP-βCTF (or C99), respectively (Figure 1). Subsequent processing by γ-secretase – a multiprotein complex [19] with either Presenilin-1 (PSEN1) or Presenilin-2 (PSEN2) as its catalytic subunit [20–22] – first releases the APP intracellular domain (AICD) while trimming the newly generated carboxy-terminus to produce peptides of varying lengths [23]. Whereas processing of APP-αCTFs yields short p3 peptides, intramembrane proteolysis of APP-βCTFs produces Aβ peptides. These are respectively referred to as the 'non-amyloidogenic' and 'amyloidogenic' pathways, with the latter being particularly prevalent in neurons relative to non-neuronal cells. However, alternative APP-CTFs can be generated: cleavage of full-length APP by the asparagine endopeptidase AEP generates APP-δCTFs that can be further processed by β- and γ-secretase, participating in the Aβ burden [24]. Alternatively, the metalloprotease MT5-MMP generates APP-ηCTFs that in turn become substrates for α- or β-secretase; the resulting Aη-α and Aη-β peptides are suggested to also disrupt neuronal health [25], possibly by modulating NMDA receptor activity [26]. Finally, meprin β can also cleave APP, generating N-terminally truncated, aggregation-prone Aβ species [27], a pathway recently linked to APP phosphorylation at Ser675 [28].

Aβ peptides typically range from 37 ($Aβ_{37}$) to 46 ($Aβ_{46}$) amino acids in length, with longer Aβ peptides (>$Aβ_{40}$) having a greater hydrophobicity and, as such, an increased tendency to self-aggregate [29]. Importantly, the site of Aβ production is primarily determined by the PSEN homolog in the γ-secretase complex [30,31]. PSEN1/γ-secretase mainly localizes at the plasma membrane and in early and recycling endosomes, from where it predominantly generates extracellular Aβ. In contrast, PSEN2/γ-secretase is restricted to late endosomes and lysosomes (LE/Lys), producing the intracellular Aβ pool [30]. Intraneuronal

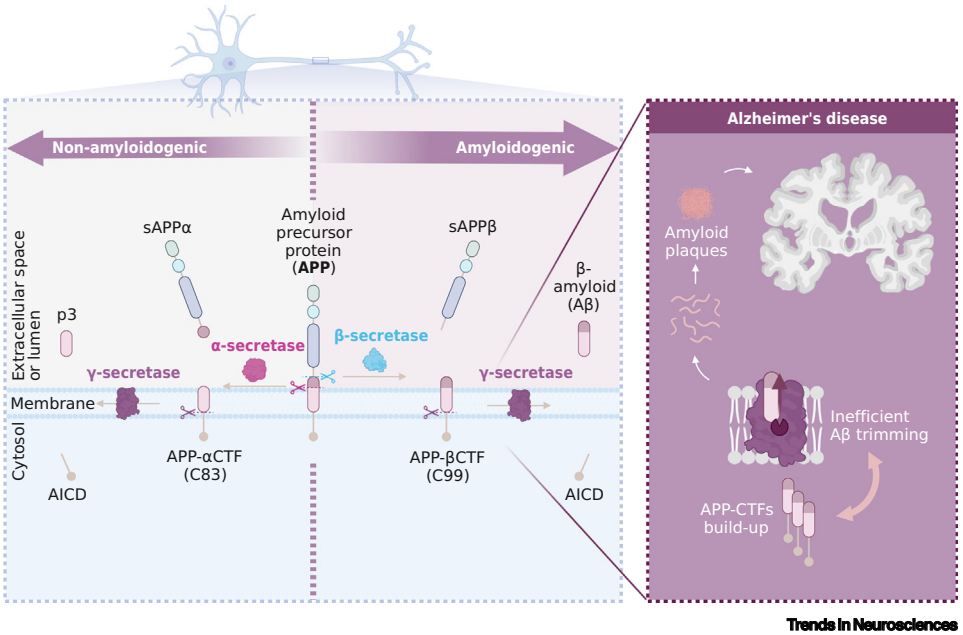

Figure 1. Proteolytic processing of the amyloid precursor protein (APP). The type I transmembrane protein APP undergoes initial cleavage by either α-secretase or β-secretase, entering the non-amyloidogenic (left) or amyloidogenic (right) pathway, respectively. In both pathways, a second proteolytic cleavage by γ-secretase releases the APP intracellular domain (AICD) into the cytosol. Cleavage of α-secretase-generated C-terminal fragments of APP (APP-αCTFs) by γ-secretase produces small p3 peptides, whereas cleavage of APP-βCTFs generates β-amyloid (Aβ) peptides of varying lengths. In Alzheimer's disease (right panel), decreased efficiency of γ-secretase processing may co-occur, leading to both the accumulation of APP-CTFs and the production of longer aggregation prone Aβ in the brain, ultimately forming amyloid plaques and driving widespread neurodegeneration. Figure created in BioRender (Vrancx, C. (2025) https://BioRender.com/0plb4il).

Aβ has been shown to progressively accumulate in AD mouse models and human brains prior to the appearance of amyloid plaques, implicating this pool in early AD pathology [32,33]. Notably, Aβ accumulates most prominently within multivesicular bodies, compromising their maturation [34]. High Aβ concentration and acidic pH likely promote toxic aggregation, triggering a pernicious cycle of organellar damage [33,34], neuronal stress, and ultimately degeneration [35,36].

Interestingly, inherited pathogenic mutations in the *PSEN1* and *PSEN2* genes decrease γ-secretase processivity [37], leading to the generation of longer Aβ peptides [38,39]. In the case of *PSEN2*, familial AD (FAD)-linked mutations strongly shift activity toward $A\beta_{42}$ production [30]. *PSEN1* mutation-driven changes in Aβ profiles correlate with the age at onset of AD, with the ratio of 'short' Aβ isoforms ($\leq A\beta_{40}$) to 'long' Aβ isoforms ($\geq A\beta_{42}$) serving as a predictor of age at onset in individuals carrying these mutations [40]. Importantly, *PSEN1* mutations that most strongly reduce processivity also relocate γ-secretase to LE/Lys, further contributing to the intracellular toxic pool of Aβ [30].

The disease-associated inefficient trimming of Aβ to shorter forms has also been attributed to a loss of function for γ-secretase [41,42]. Such failure in γ-secretase activity implies that APP metabolism becomes 'stuck' at the preceding step, leading to an accumulation of APP-CTFs. Given the prevalence of the amyloidogenic pathway in neurons, this blockade significantly affects β-secretase-derived APP-βCTFs (C99) which consequently accumulate inside neurons.

It may seem counterintuitive that in AD, both the substrates (APP-CTFs) and the products (toxic Aβ species) may accumulate and exert combined toxicities. However, recent observations suggest that elevated levels of $A\beta_{42}$ may partly inhibit γ-secretase activity, leading to the accumulation of unprocessed substrates, including APP-CTFs and other substrates [43]. This product-mediated feedback inhibition was demonstrated through kinetic analyses across cell-free, cell-based, and *ex vivo* models. Additionally, FAD mutations in *PSEN2* not only shift processing toward $A\beta_{42}$ but also lead to increased levels of APP-CTFs [44], a phenomenon similarly observed with certain FAD mutations in *PSEN1* or in *APP* [45], particularly near the γ-secretase cleavage site [46]. Local accumulation of APP-CTFs may also increase the likelihood of their homo-dimerization, a process proposed to enhance toxicity [47,48], as has been demonstrated for other γ-secretase substrates such as p75 [49]. Together, these factors create a framework for a synergistic deleterious effect of Aβ and APP-CTFs, rendering neurons vulnerable and ultimately leading to neuronal death.

In this context, it should be acknowledged that beneficial effects of γ-secretase inhibition with the potent *N*-[*N*.(3,5-difluorophenacetyl-l-alanyl)]-S-phenylglycine *t*-butyl ester (DAPT) compound have been reported in some studies [50–52], which may seem to contradict the view that APP-CTFs are toxic entities per se. However, these studies evaluated short-term treatments in young or early-stage transgenic animals, where Aβ production is acutely blocked but APP-CTFs may not have yet reached neurotoxic thresholds. Indeed, other reports have shown that chronic γ-secretase inhibition, particularly under conditions that allow for substrate buildup, does result in endolysosomal defects, synaptic impairment and neurodegeneration [53–55]. As such, the inhibition of γ-secretase to prevent AD onset may alter memory by itself through disrupting the persistence of activity-dependent synaptic plasticity [54].

Historically, APP-CTFs – particularly APP-βCTFs/C99 – have long been known to exert cellular toxicity. As early as the 1990s, a study in a C99 overexpression transgenic mouse model concluded that APP-βCTFs may be even more toxic than Aβ itself [56]. These mice exhibited a strong amyloid load, including very high Aβ plasma levels, but without accompanying neuropathology. As brain APP-βCTFs levels were eightfold lower than in either cultured overexpressing C99 neurons or the same neurons transplanted into mouse brains – both of which had demonstrated significant neurotoxicity [57,58] – it was concluded that the local degradation of APP-βCTFs in the brain was beneficial, alleviating neuronal toxicity. In agreement, a more recent knock-in mouse model carrying *APP* and *PSEN1* mutations showed APP-βCTFs accumulation that triggered early brain pathology progressively worsening with age [59]. A substantial body of evidence also demonstrated the post-mortem accumulation of APP-CTFs in human brain tissue from individuals with AD [60], Down syndrome [61] or APP locus duplication [62]. These studies support the notion that APP-CTFs are endogenously involved in AD pathology, and that their accumulation is a dose-sensitive pathogenic event.

Interestingly, post-mortem analysis of both familial and sporadic AD brains demonstrated the local accumulation of APP-βCTFs in synapses, suggesting a direct role in their degeneration [63]. Importantly, APP-βCTFs levels were shown to correlate with the degree of cognitive impairment in individuals with AD and to selectively accumulate in vulnerable neurons [12]. This contrasts with Aβ levels, which appear to increase in both vulnerable and resistant brain areas. Together, these findings lend support to the hypothesis that APP-(β)CTFs may act as early triggers of neurotoxicity in AD.

APP-CTF accumulation from genetic mutations: a catalyst for endolysosomal dysfunction

At the cellular level, proteolysis of APP-(β)CTFs occurs in all compartments where active γ-secretase resides, ranging from the cell surface to early and late endosomal compartments [64–66]. APP-CTFs that escape PSEN-mediated degradation are targeted to lysosomal and

autophagic compartments for degradation [11,67]. There is also an evident correlation between AD-linked aberrant APP proteolysis and endolysosomal abnormalities (Figure 2), which gained physiological relevance through recent studies in human cellular models. In iPSC-derived human neurons, *APP* and *PSEN1* mutations caused enlargement of Rab5-positive endosomes [68] and common defects in the lysosome-autophagy system [69], whereas Down syndrome human fibroblasts displayed unhealthy lysosomes accumulating APP-βCTFs [13].

Although these observations could be partially explained by an increased production and accumulation of toxic $A\beta_{42}$ in late endosomes akin multivesicular bodies [33,70,71], attention is now shifting towards the toxicity arising from APP-CTFs, and in particular APP-βCTFs. Indeed, in human neurons, both pharmacological inhibition of BACE1/β-secretase and knockout of APP were shown to rescue defects in early endosomes, lysosomal proteolysis, and autophagic clearance [68,69]. As these two strategies share the common mechanistic effect of lowering endogenous APP-βCTFs levels, this underlines the toxic impact of their accumulation on endolysosomal homeostasis. In support, inverse effects were observed in healthy human neurons treated with γ-secretase inhibitors, which result in vast APP-CTF accumulation [69].

Importantly, *APP* and *PSEN1* mutations were shown to exert differential effects on Aβ profiles but similar effects on APP-βCTFs, with the latter being a strict correlate of endolysosomal dysfunction [68]. Although both APP-αCTFs and APP-βCTFs have been demonstrated to compromise endolysosomal homeostasis [14], in neurons this may be largely attributed to APP-βCTFs.

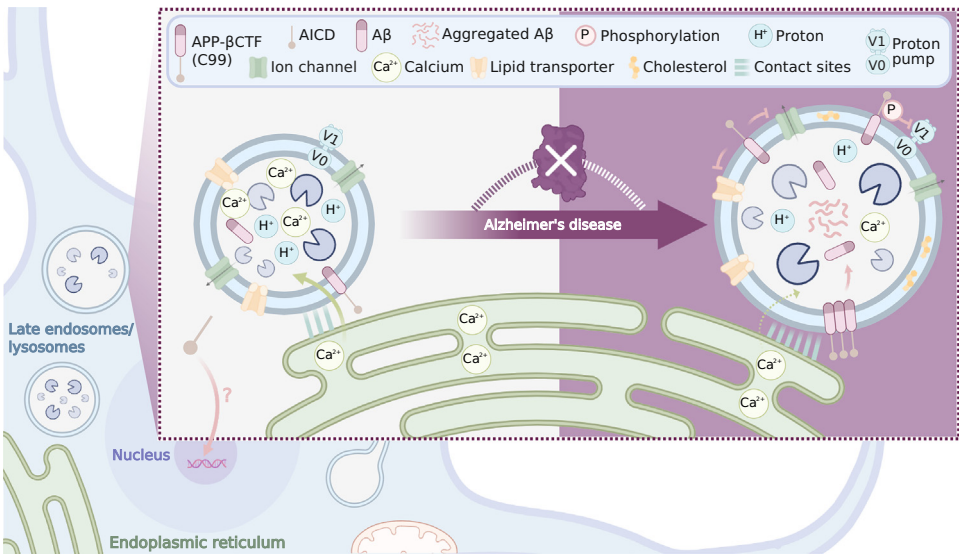

Figure 2. Amyloid precursor protein-C-terminal fragment (APP-CTF) accumulation can drive endolysosomal dysfunction. Under healthy conditions (left panel), APP-CTFs are processed within the membranes of late endosomes and lysosomes (LE/Lys), preventing their accumulation. A reasonable amount of β-amyloid (Aβ) is generated in the organelle lumen, which cells can effectively clear. Healthy organelles communicate via membrane contact sites (MCSs) and, together with receptors and channels, maintain homeostatic ion and lipid concentrations. In Alzheimer's disease (right panel), APP-CTFs accumulate within LE/Lys MCSs, while excessive untrimmed Aβ peptides aggregate in the lumen and may affect LE/Lys integrity. Together, this results in LE/Lys dysfunction and compromises inter-organellar communication through MCSs, disrupting ion balance and lipid metabolism [14]. Defective ion homeostasis involves particularly calcium and protons, with the latter linked to disrupted v-ATPase assembly caused by the interaction of phosphorylated APP-CTFs with the V0 segment [13]. Abbreviation: AICD, APP intracellular domain. Figure created in BioRender (Vrancx, C. (2025) https://BioRender.com/uglcxjg).

Moreover, in a disease context, the levels of APP-βCTFs phosphorylated at Tyr682 were shown to be augmented [13]. This residue lies within the canonical C-terminal ^{682}YENPTY^{687} motif of APP, required for internalization and endosomal sorting [72], but also for APP intracellular signaling [73]. Mechanistically, phosphorylated APP-βCTFs promoted interaction with the lysosomal v-ATPase V0 segment, disrupting its association with the V1 subunit and impairing acidification [13] (Figure 3). Inhibition of the Fyn kinase rescued v-ATPase assembly and lysosomal pH, highlighting its pathological role in APP-βCTFs toxicity. With α- and β-secretase inhibitors exerting divergent effects, APP-αCTFs were concluded to not be involved in this process, likely because they are predominantly produced at the cell surface, where a functional v-ATPase is less essential. Of note, the YENPTY motif, present in both α- and βCTFs, was suggested to induce endosomal enlargement through the recruitment of the Rab5 effector APPL1 [68,74].

Interestingly, endolysosomal defects arising from accumulating APP-CTFs were also recently identified with pharmacological inhibition or genetic ablation of γ-secretase in murine cells, both of which fully block Aβ production [14]. Time-lapse inhibition of γ-secretase revealed a temporal sequence of endolysosomal defects, with lysosomal calcium being affected first, followed by defects in recycling and early endosomes. This aligns with previous studies highlighting a lysosomal

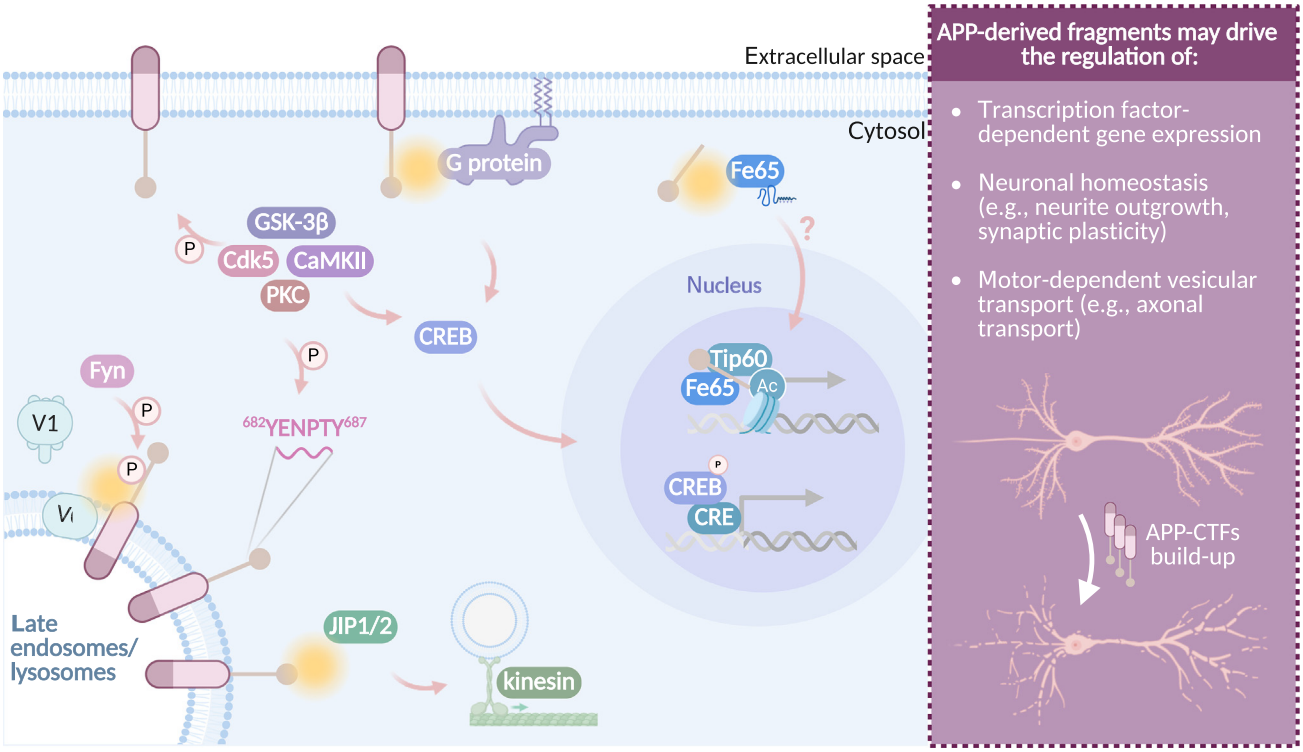

Figure 3. Molecular actors of amyloid precursor protein-C-terminal fragment (APP-CTF) toxicity. The YENPTY motif of APP can be phosphorylated by various kinases, including GSK-3β, Cdk5, CaMKII, and PKC. The latter two kinases can activate cAMP responsive element-binding protein (CREB) signaling, which may also occur upon APP-CTF binding to G-proteins at the cell surface [93]. Activated signaling leads to the transcription of genes that support neuronal homeostasis, including neurite outgrowth and synaptic plasticity. Phosphorylation of the YENPTY motif by Fyn kinase, however, promotes excessive interaction with the V0 subunit of the v-ATPase proton pump, disrupting its proper assembly [13]. APP-CTFs have also been shown to bind JIP1/2 adaptor proteins, which facilitate binding to the anterograde motor kinesin, thereby ensuring correct axonal transport [95,96]. Finally, the requirement for γ-secretase to degrade excess APP-CTFs questions the role of APP intracellular domain (AICD) forming a cytosolic complex with Fe65 and Tip60 for downstream transcriptional regulation [83]. Figure created in BioRender (Vrancx, C. (2025) https://BioRender.com/49nz81f).

calcium deficit in PSEN-deficient cells [75,76]. Overall, these and other studies point to the endolysosomal system being a primary site of insult for accumulating APP-CTFs.

A recent study using super-resolution microscopy localized APP-CTFs to membrane contact sites (MCSs) between LE/Lys and the endoplasmic reticulum (ER) [14]. MCSs are narrow inter-organellar regions where membranes are juxtaposed to facilitate the exchange of metabolites, lipids, and ions, critical for maintaining organelle homeostasis. Excessive accumulation of APP-CTFs near/in these MCSs disrupted contacts morphology, leading to deficient lysosomal Ca^{2+} refilling from the ER and lysosomal cholesterol accumulation (Figure 2), and triggering endolysosomal collapse [14]. Notably, APP knockout restored lysosomal Ca^{2+} levels and endolysosomal homeostasis, elucidating how excess APP-CTFs drive LE/Lys toxicity and highlighting the need for balanced APP-CTFs to maintain dynamic MCSs. Moreover, as MCSs are pivotal for inter-organellar communication, disturbances at these sites – such as those caused by APP-CTF accumulation – may act as conduits for propagating organellar dysfunction. While MCSs typically enhance cellular resilience, their dysfunction may render neurons vulnerable [77,78]. Substantial evidence supports endolysosomal dysfunction as a primary driver of neuronal vulnerability, which, when combined with disrupted proteostasis, culminates in synaptic dysfunction and ultimately neuronal death [79].

Protecting neurons: balancing APP-CTF levels matters

A critical question arises: why do cells, and in particular neurons, fail to tolerate excess APP-CTFs? Intramembrane proteolysis by γ-secretase is typically regarded as a mechanism to initiate downstream signaling and transcriptional activation of target genes, as exemplified by its well-known substrate, Notch [80]. In this context, ligand binding triggers sequential cleavage of Notch by ADAM and γ-secretase proteases, releasing the Notch intracellular domain (NICD). NICD then forms a transcriptionally active complex that translocates to the nucleus to activate its target genes [81,82]. Similar 'signaling by release' mechanisms are well-documented for other γ-secretase substrates, highlighting its essential role in transcriptional regulation of cellular pathways. Disruption of these pathways is associated with diseases, including cancer and neurodegeneration [80].

In the case of APP, several studies proposed a similar signaling role for the APP intracellular domain (AICD) following γ-secretase cleavage. AICD was shown to interact with Fe65 and the histone acetyltransferase Tip60 and suggested to form a nuclear complex that regulates gene expression (Figure 3), including genes implicated in cellular processes such as apoptosis, cytoskeletal dynamics, and metastasis suppression [83,84]. In addition, studies suggested that AICD regulates genes involved in APP processing itself – for example, BACE1 – hinting at a feedback loop controlling amyloidogenic pathways, as well as genes associated with synaptic receptors mediating neuronal maturation [85]. Alternatively, soluble AICD was proposed to directly interact with the glycogen synthase kinase GSK3β to promote its kinase activity, thereby inhibiting Wnt signaling at the benefit of neuronal proliferation and differentiation [86]. Finally, soluble AICD has also been proposed to modulate intraneuronal calcium homeostasis [87], with consequences for neuronal excitability [88].

Nevertheless, a signaling role for soluble AICD remains controversial, not least because most reported transcriptional effects were observed in systems overexpressing APP and/or Fe65, or relied on exogenous expression of soluble AICD itself, sometimes even tagged with a nuclear localization signal [85,88]. Several studies were also conducted in the presence of γ-secretase inhibitors to distinguish AICD effects from those mediated by Aβ, yet overlooking the concomitant dramatic accumulation of its substrate, APP-CTF. Furthermore, transgenic mouse models

overexpressing AICD and Fe65 have failed to show consistent transcriptional changes or neurodegenerative phenotypes, unless crossed with lines carrying additional AD-related mutations [89]. Notably, the molecular mechanism by which APP and Fe65 mediate transcriptional activation has been shown to depend on membrane-tethered AICD, which acts indirectly by activating Fe65 [90]. Endogenous soluble AICD was demonstrated highly unstable and rapidly degraded by the proteasome, raising further doubts about its transcriptional role *in vivo* [91].

Awaiting more physiologically relevant models to study potential roles of soluble AICD, other work has also shifted attention toward membrane-bound APP fragments as primary mediators of APP-dependent signaling, in particular linked to neuronal functions. For instance, interaction with Abl kinase induces axonal arborization, as shown for both human APP and its *Drosophila* ortholog, APP-like (APPL) [92]. Interestingly, inhibiting Abl kinase through Imatinib significantly rescued endolysosomal defects in PSEN deficient cells [14]. APP-CTFs were also shown to bind and activate heterotrimeric G-proteins, thereby triggering cAMP responsive element-binding protein (CREB)-dependent signaling, with downstream effects on neuronal morphology, synaptic plasticity, and dendritic growth [93] (Figure 3). Membrane anchoring of AICD via myristoylation was necessary and sufficient to trigger signaling. A similar requirement for membrane-anchoring of AICD was observed in relation to the role of APP-CTFs in endolysosomal homeostasis [14], further supporting the physiological and functional significance of membrane-bound APP fragments.

Overall, these studies support the idea that, unlike for Notch, γ-secretase-mediated cleavage of APP-CTFs may de-activate signaling of its cytosolic domain. Whether the proteolysis of other substrates, such as p75, also leads to termination of their signaling function remains to be further investigated.

By eliminating APP-CTFs at late endosomes/lysosomes-endoplasmic reticulum (LE/Lys-ER) MCSs, γ-secretase safeguards lysosomal Ca^{2+} levels, which are critical for lysosomal function [14,94]. This protective role may prevent prolonged interactions between the cytosolic domain of APP-CTFs and kinases or adaptor proteins, offering an alternative mechanism for regulating APP-associated downstream signaling. Indeed, multiple kinases – GSK-3β, Cdk5, PKC, and CaMKII – phosphorylate APP, influencing its trafficking and interactions with adaptors such as Fe65, X11L, or the kinesin-binding proteins JIP1/2 [95,96] (Figure 3). These adaptors, in turn, regulate processes like gene expression, axonal transport, neurite outgrowth, and synaptic plasticity. Mechanisms that lead to APP-CTF accumulation could thus disrupt the delicate balance of signaling events, underscoring the critical role of γ-secretase in cleaving and degrading these fragments to maintain cellular homeostasis.

Dysregulated APP trafficking/proteolysis or endolysosomal dysfunction: which comes first?

Endolysosomal defects are among the earliest preclinical signs of AD pathology [16,97]. Increasing evidence links the aberrant accumulation of APP processing fragments in endosomal and lysosomal compartments to the onset and progression of AD. While much of this evidence comes from studies of FAD-linked mutations in *APP*, *PSEN1*, and *PSEN2*, as discussed earlier, similar disruptions in APP trafficking and processing within the endolysosomal system are observed in sporadic or late-onset AD (LOAD). These disturbances may therefore be driven by genetic polymorphisms and other risk factors [98,99].

Genome-wide association studies have identified 101 independent AD-associated single-nucleotide polymorphisms (SNPs) across 81 loci [100]. Over 500 variants have been identified in the *SORL1* gene, which encodes the neuronal receptor SORLA, a key regulator of APP trafficking [101]. While SORLA haploinsufficiency causes endosomal dysfunction, complete loss further

impairs lysosomal function and autophagy. Notably, reducing APP expression using antisense oligonucleotides mitigates endolysosomal dysfunction caused by SORLA loss [102], alike observed in PSENdKO cells [14].

Variations in apolipoprotein E (ApoE) also stand out due to ApoE's critical role in cholesterol homeostasis [103]. ApoE isoforms differentially influence APP processing in human neurons, with the ApoE4 isoform – associated with increased AD risk – enhancing amyloidogenic APP processing and elevating Aβ production [104].

Other endosomal AD risk genes include *CD2AP, BIN1*, and *PICALM* [105,106]. CD2AP deficiency impaired the sorting of axonal APP into intraluminal vesicles of multivesicular bodies in mouse neuronal cells, increasing amyloidogenic APP processing [107]. BIN1 knockout in murine neuronal cells disrupted the recycling of BACE1/β-secretase to the plasma membrane, leading to its accumulation in early endosomes and promoting amyloidogenic processing [107]. In neuronal-like cells and mouse brains, PICALM was found to influence APP processing by facilitating γ-secretase internalization [108] and modulating BACE1/β-secretase expression [109].

Lysosomal proteins encoded by AD risk genes also play critical roles in APP processing. PLD3, a lysosomal exonuclease, degrades CpG-rich mitochondrial DNA delivered via mitophagy [110,111]. LOAD-associated SNPs in *PLD3* reduce its activity, causing lysosomal APP-CTF accumulation and activation of the cGAS-STING signaling pathway, further amplifying dysfunction and resulting in a degradative bottleneck [111].

Collectively, these studies demonstrate that endolysosomal defects are closely tied to APP processing and can often be alleviated by reducing APP expression. This underscores a direct link between AD – both familial and sporadic – and endolysosomal dysfunction, highlighting this system as a critical site of toxic fragment initiation [71], including not only Aβ but also APP-(β)CTFs.

Notably, PSEN/γ-secretase activity is also closely linked to the regulation of lipid metabolism [112]. For instance, inactivating γ-secretase, either pharmacologically or genetically, reduced neuronal cholesterol levels, impairing synaptic function in iPSC-derived human neurons [113]. Cholesterol transport relies heavily on endolysosomal function, and studies have demonstrated that altering neuronal cholesterol impacts γ-secretase-dependent APP processing. These findings reinforce the interconnectedness of lipid metabolism and endolysosomal transport, both of which are vulnerable pathways in AD [106].

APP-CTFs likely act in concert with other proteolytic fragments to contribute to endolysosomal dysfunction. Intracellular $A\beta_{42}$ peptides were shown to accumulate in late endosomes and multivesicular bodies, where they exert toxicity [34,70]. When exogenously administered, $A\beta_{42}$ is similarly targeted to LE/Lys, where low pH and ion content drive fibril formation, causing organelle damage [114]. This supports a 'double-hit model' in which APP-CTFs and $A\beta_{42}$ fragments cause chronic damage to the endolysosomal system through distinct mechanisms – sustained signaling and physical damage, respectively – likely overlapping in early stages of AD. In support, a knock-in FAD-PSEN2 mouse model showed a concomitant increase in $A\beta_{42}$ and APP-CTFs, which caused endolysosomal dysfunction and led to working memory deficits through reduced long-term potentiation in the hippocampal CA3 region [44]. Cellular aging mechanisms may further accelerate these processes. In neurons and brain tissue from 5xFAD mice crossed with telomerase-deficient senescent mice, accelerated senescence reduced autophagic flux, leading to early intraneuronal amyloid accumulation and neuronal death [115]. This ties lysosomal-autophagy defects and aberrant APP

proteolysis to aging, AD's key risk factor. Interestingly, cognitively healthy centenarians exhibit a lower polygenic risk score and are gradually depleted of AD-associated loci while enriched with protective loci [116]. This reduced polygenic risk score underscores a genetic protection against AD [117]. Notably, some of the strongest enrichments and depletions were observed in genes associated with the endolysosomal system, such as *RIN3*, *TMEM106B*, *GRN*, and *SORT1*. These findings suggest that maintaining cognitive health may depend on the proper functioning of the endolysosomal system.

Concluding remarks and future perspectives

The studies discussed here support a model in which defective PSEN/γ-secretase activity in a disease context may lead to excess APP-(β)CTFs, particularly within the endolysosomal system. This accumulation impairs organellar homeostasis and communication, triggering cellular stress. Thus, APP-(β)CTFs should be considered as an additional contributor to AD onset and progression, and represent a potential therapeutic target – particularly for alleviating endolysosomal defects and restoring inter-organellar communication. Of note, the heightened toxicity of APP-βCTFs compared to APP-αCTFs may be attributed to their distinct sites of generation. APP-βCTFs are preferentially retained within endolysosomes, where their accumulation and local interactions with other proteins may promote organelle dysfunction. Such interactions, including with proteases such as BACE1 [118] or components of the trafficking machinery, could contribute to impaired organellar homeostasis and downstream pathological effects.

Mounting evidence in FAD- and Down syndrome-related models supports the notion of limited tolerance for excess APP-CTFs in maintaining cellular balance, underscoring a physiologically relevant role for γ-secretase activity in terminating APP-CTF-dependent signaling. Future work should focus on developing both theoretical and experimental strategies to identify the precise downstream signaling cascades of APP-CTFs, particularly from their restricted location at LE/Lys-ER MCSs (see Outstanding questions).

The presence of APP-CTFs at LE/Lys-ER MCSs implies nearby activity of both BACE1 and γ-secretase, pointing to a yet largely unexplored role for intramembrane proteolysis in inter-organellar communication by regulating substrate levels within MCSs. Notably, studies have identified APP-CTFs and PSEN2 in mitochondria-associated membranes [119–121]. However, in more recent work from our group using super-resolution microscopy we were unable to confirm these observations [14], and additional research will be needed to address these discrepancies. In our view, the localization of APP-CTFs in mitochondria-associated membranes seems unlikely given that (i) γ-secretase assembly and activation occurs after ER exit [122], and (ii) the prevailing consensus is that the dual processing of APP by sheddases and γ-secretase is largely a post-Golgi event. Instead, we postulate that the observed mitochondrial defects in the context of γ-secretase dysfunction (or, by extension, in a FAD context) may be indirect, originating from defective or damaged late endosomes/lysosomes and propagating defects to mitochondria, potentially through MCSs. This could explain the co-occurrence of lysosomal and mitochondrial dysfunctions in early, preclinical stages of AD.

Importantly, the studies reviewed here underscore that it could be beneficial for future therapeutic approaches to address both APP-CTFs and Aβ to restore the balance between these fragments. For instance, γ-secretase modulators [123] and allosteric stabilizers [55] are designed to restore processing activity, which should result in the reduction of both long, toxic Aβ peptides and APP-CTFs. In parallel, while the approach of broad BACE1 inhibition has largely failed due to toxicity, low-dose inhibition has recently been re-examined to modestly reduce both Aβ and APP-βCTFs [124]. However, translating these insights into meaningful clinical interventions is challenging.

Outstanding questions

The accumulation of APP CTFs appears to be an early trigger of organellar and neuronal dysfunction. Could pharmacological interventions effectively mitigate the toxicity of these fragments?

Could therapeutic strategies targeting the proteolytic pathway of APP processing selectively reduce APP-CTFs and toxic Aβ levels while preserving the putative physiological functions of APP?

What potential biomarkers are associated with APP-CTF toxicity, and can they be leveraged for early diagnosis and treatment?

How do deficits in intramembrane proteolysis at inter-organellar contact sites mechanistically contribute to organellar homeostasis as an early driver in neurodegenerative disease pathology?

To what extent do APP-CTFs contribute to tau hyperphosphorylation and its downstream effects on neuronal function?

How do APP-CTFs interact with glial cells, particularly microglia, to influence neuroinflammation and exacerbate AD pathology?

How does aging, the most prominent risk factor for AD, impact APP metabolism and APP-CTFs levels?

Therapeutic approaches should be selectively targeted to modulate APP processing and spare other substrates. This is critical as the failed clinical trials using γ-secretase inhibitors clearly highlighted concerns about specificity, on-target toxicity, and adverse cognitive effects.

From the perspective of gene therapy, the introduction of a disease-protective variant – such as the Icelandic A673T mutation in *APP* – could be a promising approach for individuals at high risk for AD. A proof of concept was recently shown in a preclinical AD mouse model harboring the A673T mutation [125]. This model showed reduced β-secretase processing, leading to reduced amyloid pathology and neuroinflammation [125]. Although appealing conceptually, *in vivo* gene editing faces delivery challenges and unresolved ethical considerations [126].

Direct APP downregulation may offer a more realistic and scalable approach to reduce all types of proteolytic fragments. Antisense oligonucleotides have shown promise in preclinical models by rescuing endolysosome and autophagy dysfunction [102]. In parallel, siRNA-based strategies like ALN-APP – mivelsiran, designed with Alnylam's C16-siRNA technology to enhance delivery to brain cells – are under clinical investigation and offer an alternative APP silencing modality with distinct pharmacokinetics and delivery profiles [127]. Given APP's essential roles, titrated or neuron-specific knockdown may be needed to avoid adverse effects. Overall, future therapeutic strategies targeting APP/amyloid remain challenging, requiring efficient brain penetrance and neuronal delivery, while minimizing off-target effects and ensuring overall safety to advance toward clinical translation.

On a final note, a body of literature has demonstrated more direct, non-catalytic roles for PSENs in lysosomal and autophagy homeostasis – for instance, in regulating calcium levels, v-ATPase-dependent lysosomal acidification, and autophagic flux – independently of APP cleavage [16]. Loss of these non-catalytic functions may therefore contribute to disease even in the absence of substantial APP-CTF accumulation. Moreover, as discussed in the context of sporadic AD, there is a strong genetic basis placing lysosomal and autophagy dysfunction at the center of etiopathogenesis, with many risk loci linked to endolysosomal genes. Thus, given the current mixed success of amyloid-targeting strategies, therapies aimed at improving lysosomal and autophagy function could represent complementary or alternative approaches. One promising example is Blarcamesine, a sigma-1 and muscarinic receptor agonist, which enhances waste clearance through autophagy. A recent Phase 2B/3 trial in early AD reported a 36% slowing of clinical progression – outperforming Lecanemab – alongside reduced brain atrophy and no amyloid-related imaging abnormalities [128], offering promise for (combined) therapeutic intervention. Together, advances in deciphering APP-CTF-related mechanisms and growing efforts to target endolysosomal dysfunction through complementary pathways may provide avenues toward the development of safer and more effective disease-modifying therapies for Alzheimer's disease.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT in order to enhance its readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of interests

The authors declare no competing interests.

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