Journal of Neurology & Experimental Neuroscience

https://doi.org/10.17756/jnen.2019-045

Review Article

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# The Amyloid Precursor Protein: More than Just Amyloid-Beta

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**Received:** December 06, 2018
**Accepted:** January 08, 2019
**Published:** January 10, 2019

**Citation:** Jevtic S, Provias J. 2019. The Amyloid Precursor Protein: More than just Amyloid-Beta. *J Neurol Exp Neurosci* 5(1): 1-11.



Published by United Scientific Group

## Abstract

Alzheimer disease (AD) is currently the leading cause of dementia worldwide with no disease modifying therapy available. Despite the burden of disease, there is still no consensus on the underlying pathophysiology leading to sporadic AD. The previously held amyloid-beta (Aβ) hypothesis is giving way to a multi-factorial model that involves various pathologic components, including Aβ and tau. In this paradigm, other components including inflammatory processes (i.e. microglia), structural components (blood brain barrier), and the amyloid precursor protein (APP) play significant roles in disease. We present here a review on the emerging role of APP in AD, independent of Aβ, including its significance in normal physiology and disease. We briefly describe the various isoforms of APP, its processing pathways, and how this contributes to function. Furthermore, we present our own observations and discussion on APP pathology in AD human tissue. Finally, we combine this knowledge of APP to suggest an interaction with tau that could be applied to AD and other tauopathies such as chronic traumatic encephalopathy. This emerging view of APP places it as an independent-mediator of AD pathology with implications for future research and treatment avenues.

## Keywords

Amyloid precursor protein, Alzheimer disease, Amyloid-beta, Neurodegeneration, Hypothesis

## Abbreviations

Aβ: Amyloid-beta; AD: Alzheimer Disease; APP: Amyloid Precursor Protein

## Introduction

Alzheimer disease (AD) is the most common cause of dementia and affects 46.8 million people worldwide as of 2015 [1]. Given an aging population, this represents a significant impact to healthcare systems and society, both financially and emotionally. The current theory of AD pathogenesis states that the amyloid-beta (Aβ) peptide, a product of amyloid precursor protein (APP) cleavage, is the initiating pathologic factor [2, 3]. However, there is currently poor correlation of Aβ plaque load and cognitive decline in human studies [4-7]. Furthermore, over one hundred AD-targeted clinical trials have been initiated, but to date have failed to yield disease modifying therapy [8, 9]. This suggests that there are additional mechanisms promoting AD progression, which may be directly or indirectly linked to Aβ.

One relatively unexplored avenue in AD involves focusing on APP, the precursor to Aβ peptide production. While many studies have targeted the pathways involved in Aβ formation, few have investigated normal APP physiology and its independent role in AD. Here we provide a comprehensive review of relevant APP studies focusing on its function in both homeostatic and pathologic states. We also present interesting case studies with our own observations that support a unique role of APP in AD pathogenesis. This information will seek to clarify further aspects of this disease in the hopes of finding novel avenues for therapeutic targets.

## APP Structure, Processing, and Location

APP is a type-I transmembrane glycoprotein that contains a large extracellular domain with a short cytoplasmic tail [10-12]. The APP gene is located on the long arm of chromosome 21 and contains 18 exons that are alternatively spliced. It belongs to the family of proteins including APP-like protein 1 (APLP1) and APLP2, many of which are conserved across several species including invertebrate *C. elegans* and *D. melanogasters* [13-15]. Interestingly, APLP-1 is found only in mammals and lacks two exons found in both APP and APLP-2, suggesting that it diverged during evolution from the latter gene [16]. Of the three genes, only APP contains the motif required for formation of the Aβ peptide. The APP splice variants range from 365-770 amino acids with three major Aβ-containing isoforms: APP695, APP751, and APP770, with the number indicating amino acid length [16].

APP family members share various commonalities, including structure and function, but also harbor unique differences. For example, APLP-1 is expressed exclusively in the brain while APP and APLP-2 are found in multiple tissues (including thymus, heart, muscle, etc.) [17]. Of the three major isoforms of APP, APP-695 is most predominant in the brain and expressed almost exclusively in neurons [17]. In terms of protein composition, all members of the APP family contain extracellular dimerization domains known as E1 and E2 and share conserved regions in their C-terminal domains. However, the N-termini are more variable; for example, APLP2 and APP770 contain a Kunitz protease inhibitor region and OX-2 domain which are lacking in APP695 and APLP-1 [18]. These conserved regions likely confer overlapping functions while N-terminal variability offers unique properties to correlate with tissue distribution.

Once it has been translated, APP can undergo multiple processing pathways to yield various peptide products (Table 1). The most well-studied of these are the non-amyloidogenic and amyloidogenic pathways, involving α- and β-secretase, respectively. Both pathways cleave APP in the extracellular domain but at different locations and using different enzymes.

### α-secretase pathway

α-secretase, most commonly the ADAM-10 metalloproteinase, cleaves APP to yield a soluble APPα (APPsα) fragment [19, 20]. Since α-secretase cleaves in the Aβ domain, this pathway precludes formation of Aβ peptide. The α-secretase pathways also moderates additional APP functions, such as cell-adhesion, whereby cleavage leads to diminished activity (see Function below). The remaining APP protein is cleaved by γ-secretase (common to α and β pathways); the γ-secretase complex is composed of presenilin (including PSEN1 and 2), nicastrin, anterior pharynx defective 1, and presenilin enhancer 2 subunits [21]. Together they cleave the remaining C-terminal fragments yielding an intracellular C-domain (AICD) and p3 peptide (Table 1).

**Table 1:** APP Processing Pathways and Potential Products. Pathways are listed with key enzyme mediators in order of APP substrate cleavage.

| Pathway | Mediators | Substrate | Products |
|---|---|---|---|
| Amyloidogenic | β-secretase | APP | APPsβ<br>C99 |
| | γ-secretase | C99 | $Aβ_{1,37-42}$<br>AICD |
| Non-amyloidogenic | α-secretase | APP | APPsα<br>C83 |
| | γ-secretase | C83 | p3<br>AICD |
| δ-secretase | AEP | APP | $APPsδ_{1-585}$/$APPsδ_{1-373}$ + $APPsδ_{374-585}$<br>CTFδ |
| | β- and γ-secretase | CTFδ | Aβ<br>AICD |
| η-secretase | MT5-MMP | APP | APPsη<br>CTFη |
| | α-secretase | CTFη | Aη-α<br>CTFα |
| | β-secretase | CTFη | Aη-β<br>CTFβ (contains Aβ sequence intact) |
| Meprin | Meprin β | APP | $APPs_{1-124}$<br>$APPs_{1-305}$/$APPs_{1-308}$<br>$^{Δ}$APPsβ* (1 additional AA)<br>$^{Δ}$CTFβ* (1 fewer AA) |
| | γ-secretase | CTFβ* | Aβ* |
| Caspase | Caspase | APP | APP-ΔC31<br>C31 |
| | | CTFα/β | CTFα/β-ΔC31<br>C31 |
| | γ-secretase | CTFα/β-ΔC31 | p3/Aβ<br>Jcasp |

**Note:** ΔAPPsβ* contains one additional AA compared to regular APPsβ; CTFβ* and Aβ*contain one fewer. AA = amino acid.

### β-secretase pathway

β-secretase (also known as BACE1 or BACE2) initially produces both a soluble APPβ fragment (APPsβ), which is 16 amino acids shorter than APPsα, and a membrane-bound C-terminal fragment of 99 residues (C99) [22]. Despite sharing a similar amino acid sequence, APPsα and β peptides yield markedly different structures and function [11, 12, 23]. C99 is further cleaved by the γ-secretase to yield

an intracellular C-domain (AICD) and Aβ peptides, ranging from 37-42 amino acids in length.

These canonical pathways are likely in competition for the APP substrate, whereby upregulation of one pathway will decrease products from the other. For example, activation of NMDA receptors in mouse primary cortical neurons stimulated α-secretase activity and decreased Aβ production [24]. Therefore, therapies that enhance non-amyloidogenic processing may serve as a therapeutic target.

In addition to the above canonical pathways, several non-canonical pathways have been identified, including: δ-secretase, η-secretase, meprin, and caspase cleavage pathways.

### δ-secretase pathway

Mediated by asparagine endopeptidase (AEP), a cysteine protease, which is localized to lysosomes [25-27]. It cleaves following asparagine residues and can produce three possible soluble forms (one large or two smaller products) and a CTF fragment. This CTF fragment can go on to be cleaved by β- and γ-secretase [10]. AEP has been shown to interact with APP in the endolysosomal network to produce an N-terminal fragment, of which the synthetic analogue causes neurotoxicity *in vitro* [28]. Knock-out of AEP in 5xFAD mice rescued synaptic dysfunction including LTP deficits, while pharmacological blockade reduced tau and APP cleavage in P301S and 5xFAD mice, respectively [28, 29]. Therefore, the δ-secretase pathway may produce toxic products that contribute to AD, although the extent of involvement remains unknown.

### η-secretase pathway

Mediated by MT5-MMP (membrane-type 5-matrix metalloproteinase) [30, 31]. This pathway yields a CTF-η fragment which can be further processed by α- and β-secretases to yield Aη-α and Aη-β fragments, respectively. Aη-α production increases following administration of a BACE-1 inhibitor and has been shown to reduce long-term potentiation (LTP) in mice [30]. MT5-MMP knockout in 5xFAD mice also significantly reduced Aβ production and reactive gliosis, ultimately supporting an important role in APP metabolism [32, 33].

### Meprin and caspase pathways

These pathways yield novel N-terminal fragments and C-terminal fragments, respectively [34, 35]. The meprin pathway involves meprin β metalloprotease and has been confirmed in cell models to contribute to Aβ generation, while caspase produces a neurotoxic C-terminal product (C31) that can induce cell death [36-38]. C31 toxicity was found to be dependent on APP *in vitro*, while exposure to Aβ triggered C31 production [37]. Interestingly, the converse peptide of caspase cleavage (APP-Δ31) has also been shown to be neurotoxic. Overexpression of wild-type APP in human NT2 cells results in caspase-3 activation, leading to APP-Δ31 formation and post-mitotic neuron degeneration [39, 40]. Similarly, overexpression of solely APP-Δ31 triggered neuronal death without caspase 3 activation [40]. Although these proteins have also been identified in human AD tissue, there is sparse evidence for a direct role in pathogenesis.

In addition to cleavage products, the cellular localization of APP plays an important role in its processing and function within the neuron. APP is found in somatodendritic and axonal compartments in both animal model and human tissue [41]. It is initially produced in the endoplasmic reticulum from where it travels to the Golgi network and enters the secretory pathway via Golgi-derived vesicles [42]. From there it continues to the cell surface and can be re-internalized to the intracellular compartment [43, 44]. APP expressed on the cell surface undergoes processing via the α-secretase pathway, thus preventing Aβ formation [45]. However, internalization and retrograde trafficking can lead to fusion with acidic recycling endosomes containing BACE1; this results in APP cleavage producing Aβ [43, 46]. Stimulation by glycine of cultured neurons expressing APP:GFP and BACE-1:mCherry led to increased co-localization of these proteins, suggesting an activity-dependent processing [44]. Therefore, it appears that generation of Aβ from APP is mediated by a careful balance between cell surface expression and fusion with BACE-1 containing vesicles that is partially influenced by neuronal activity.

## Function

The majority of APP research to date has focused on its relation to Aβ production. However, there is growing interest in the role of APP and its by-products during normal physiology and potentially as a direct mediator of AD pathology, independent of Aβ.

One important role of APP in normal physiology involves formation of proper synapse interactions, particularly involving the neuromuscular junction. At the cell membrane, APP forms *cis* (same cell) and *trans* (opposite cell) dimers between family members. These are primarily mediated by the extracellular E1 and E2 domains allowing formation of antiparallel dimers [47, 48]. In particular, E1 appears to be crucial in mediating the interaction between all three family members, which has been evidenced both *in vitro* and *in vivo* [49, 50]. This appears particularly crucial at neuromuscular junctions, since double knock-out of APP and APLP2 in mice generates aberrant junction morphology [51, 52]. This presents as excessive nerve terminal sprouting and postsynaptic acetylcholine receptor clusters. Both pre- and post-synaptic APP is required as shown in conditional Cre-lox knockout mice; knock-out using a neuronal rat nestin (pre-synpatic) or muscle creatine kinase (post-synaptic) promoter both lead to neuromuscular defects [52]. Therefore, APP family members can form various homo- and hetero-dimers that function to regulate nerve terminal growth and synapse morphology.

APP has also been shown to interact with multiple other synaptic proteins involved in pre-synaptic membrane homeostasis. Fractionation experiments in rat and mouse brains isolated APP in pre-synaptic plasma membrane fractions but not in free synaptic vesicles, suggesting that it is localized to the membrane portion of the presynaptic active

zone [53-55]. Loss of APP in mice, either through single or conditional double knock-out (NexCreAPP/APLP2), also alters the presynaptic proteome [55]. This conditional double knock-out bypasses the embryonic lethality associated with a double knockout by inducing gene deletion following birth. The presynaptic proteome changes observed were different between the single and conditional knock-outs: for example, there was a more significant downregulation of α-synuclein (the pre-synaptic SNARE complex protein) in double- compared to single-knockout. Together, these studies support a potentially redundant role of APP in the pre-synaptic zone. Furthermore, APP appears to interact with various receptors including: high-affinity choline transporter, integrins, the NMDA receptor, and GABAB receptors [56-60]. Interaction with the GABAergic system has been shown to alter inhibitory tone in mice, thus regulating hippocampal neurogenesis [59, 61]. Lastly, APP may be involved in cell adhesion and motility through interaction with extracellular material. *In vitro* experiments tested the ability of APP-/- mouse hippocampal neurons to adhere and grow on various extracellular matrices. These demonstrated an independent role of APP in growth cone phenotype [62, 63]. Therefore, it appears that the APP interacts with multiple proteins involved in various functions, from synaptic neurotransmission to cell adhesion.

An emerging interest in APP physiology has been the function of APPsα, which is a soluble product derived from α-secretase pathway processing (Table 1). This peptide has neuroprotective effects and is able to rescue deficits in APP knock-out mice, including: reductions in body and brain weight, grip strength, spatial learning and long-term potentiation (LTP) [64]. In both *in vitro* (SH-SY5Y cells) and *in vivo* (hippocampal slice culture) models, APPsα administration exerts anti-apoptotic effects. This was abrogated by knock-down/knock-out of APP, suggesting that full-length APP was required for this effect [65]. This APP-dependent survival pathway appears to function through G-protein-coupled activation of Akt pathway [65]. Increasing concentrations of APPsα correlated with increased Akt activity that was abolished by APP knock-down. Interestingly, in mice lacking both APP/APLP2, APPsα administration still improved LTP and synaptic density deficits [23, 66]. This double-knockout study suggests that there are also APP-independent signaling pathways involved. APPsα thus appears to function in a neuroprotective fashion through both APP-dependent and -independent signaling pathways.

These neuroprotective effects of APPsα have also been shown to modulate neuropathology and rescue memory deficits in AD mouse models. APP/PS1 male mice administered lentivirus-expressing APPsα prior to pathology onset (4 months of age) showed improvements in spatial learning and memory with no change in Aβ load [67]. When injected following onset of plaque load (10 months), APPsα rescued LTP deficits seen at 13 months, suggesting an acute benefit. Similarly in aged (12 month) APP/PS1ΔE9 mouse, injection of adeno-associated virus expressing APPsα rescued spatial memory recorded at 14 months, despite extensive plaque load [68]. These mice also demonstrated improved synaptic plasticity and LTP that was indistinguishable from littermate controls. Immunohistochemical analysis also revealed a modest but statistically significant improvement in plaque load (approximately 4% surface area decrease in hippocampus and cortex) and Iba-1+ cells surrounding plaques (increase of 5%) [68]. Interestingly, one mechanism by which APPsα appears to reduce Aβ load is through BACE1 binding that inhibits amyloidogenic processing [69]. Antibody treatment targeting endogenous APPsα in transgenic mice increased Aβ, further confirming a protective effect of APPsα [69]. Finally, APPsα also plays a role in altering tau metabolism. In SH-SY5Y and HeLa/tau cells overexpressing BACE1, APPsα treatment increased phosphorylation of GSK3β kinase and subsequently reduced tau phosphorylation [70]. GSK3β phosphorylation has previously been shown to result in kinase inactivation, suggesting that APPsα inhibits kinase activity [71]. These results were also replicated in AD mice over-expressing APPsα. Administration of a γ-secretase inhibitor had no effect suggesting that reduction in tau phosphorylation was independent of decreased Aβ production. Interestingly, β-secretase inhibition alone also increased GSK3β phosphorylation, supporting the model that APPsα reduces tau phosphorylation via BACE1 inhibition [70]. It thus seems that APPsα can yield cognitive and neuropathological benefits, at both early and later stages of disease onset. While this may be beneficial in AD therapy, the effect will likely be minimal and require additional targets/concomitant therapy.

APP is now well-established as a crucial protein in various physiologic processes, including synapse morphology and presynaptic membrane homeostasis. Processing through the α-secretase pathway also produces a neuroprotective peptide, APPsα, that may play a role in AD pathology and serve as a treatment modality. As more research emerges regarding APP function there will likely be a greater emphasis on its role in AD independent of Aβ.

## Neurodegeneration – Alzheimer Disease

The role of APP in neurodegenerative diseases such as AD has long focused on its relation to Aβ generation. Initial studies in AD pathogenesis identified various mutations in APP and the γ-secretase complex associated with familial AD [72-74]. This, in conjunction with the presence of Aβ plaques and knowledge that Aβ derives from APP, led to formation of the Aβ hypothesis [2]. Various reviews have summarized the pathophysiology of Aβ and thus we will offer only a brief discussion, with a greater focus on APP independent from Aβ.

As previously mentioned (see section APP Structure, Processing, and Location and table 1) the amyloidogenic pathway consists of APP cleavage by β-secretase (BACE1/2) followed by γ-secretase [22, 75]. Generation of the Aβ peptide leads to oligomer formation and eventually insoluble plaques, with the former species producing the greatest neurotoxicity [76-80]. While Aβ plaque formation is one of the hallmarks of AD neuropathology, it has recently been confirmed that cognitive decline clinically correlates with onset of tau pathology as shown by PET scanning [81-86]. Furthermore,

all clinical trials to date testing therapeutics to decrease Aβ production or levels in the brain have failed to produce clinically significant results [8, 87]. Although these failures are likely a result of several factors, including poor trial design and patient selection, it is suggestive that there are other disease factors that need to be considered, and thus a new focus on the pathophysiology of AD is likely required.

APP therefore emerges as a potential candidate for therapeutic targets due to its intimate involvement in AD pathophysiology. Aβ has been shown to bind APP on the plasma membrane in rat hippocampal neurons, suggesting that it may bind APP through a peptide-receptor interaction [88]. In addition, Aβ can increase APP homodimer formation at the plasma membrane, both *in vitro* and *in vivo* [89]. While the significance of this interaction remains to be further investigated, it does suggest a critical involvement of APP in AD. Furthermore, several studies have shown that the toxicity of human AD brain is partly dependent on APP. In mice, injection of human AD brain extract reduces LTP in wild type, but not APP deficient, mice [89, 90]. Administration of purified oligomer Aβ or tau to APP-KO mice also failed to induce expected memory impairments [91]. Therefore, it seems that APP is partly required for the neurotoxicity of AD, independent from Aβ production.

Independent of Aβ, APP may contribute to AD pathogenesis through loss of physiologic function and gain of pathologic function. As previously described, APP produces soluble factors that are crucial in neuronal survival following injury (see Function, section on sAPP) [92-95]. Loss of these may thus initiate or accelerate disease. Additionally, prior neuropathology investigations identified APP plaques in human brains with granular and intracellular appearance [96]. These plaques have been suggested to reside within dystrophic neurites and occasionally associate with senile plaques and/or tau staining [97-105]. More specifically, electron microscopy identified APP accumulations that associated with supposed lysosomal bodies in these dystrophic neurites [100]. However, not all of these APP aggregations co-localize with typical Aβ and/or tau staining, which is also what we see in our own cases (Figures 1-3). These APP structures have various morphologies from point-like and granular to a more diffuse appearance (Figure 3). We also confirm that these APP structures occasionally associate with typical Aβ plaques and dystrophic neurites, as labelled by tau, but are also found independently of both these hallmarks (Figure 1 and 2). Interestingly, these plaques also appear to be extracellular, suggesting that they may be a unique pathologic entity. There is currently a paucity of literature regarding these punctate APP aggregations that are independent of classical AD hallmarks. We put forth the idea that APP aggregates early on in disease within dystrophic neurites but is potentially released extracellularly with as yet unclarified contributions to disease pathology. Ultimately, although some of these APP structures may colocalize with dystrophic neurites as previously described, we suggest there is greater heterogeneity that may yield further insights into AD pathology.

To support this, there is evidence in the Tg19959 mouse model of APP changes that pre-date plaque formation. This model expresses APP with two familial mutations resulting in plaque deposition by three months. At two months, blue native page of mouse brain homogenates demonstrates high molecular weight APP species [106]. This potentially represents interaction/aggregation of APP that occurs prior to plaque deposition and serves as an initiating event. In conjunction with this model, recent studies have identified a link between APP metabolism and tau protein levels in human cortical neurons (iPSCs derived from individuals with familial AD

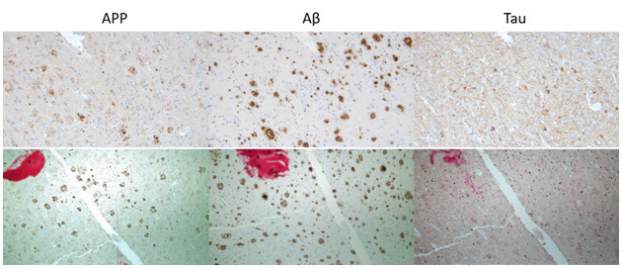


**Figure 1:** APP staining in relation to Aβ and Tau. There appears to be a poor correlation between presence of APP accumulations and Aβ/tau, as previously noted. Although occasional co-localizations occur, they do not appear to be consistent. Top row: 66 year old male, left superior temporal lobe – Lewy body dementia. Bottom row: 52 year old female, left amygdala – Frontotemporal dementia.

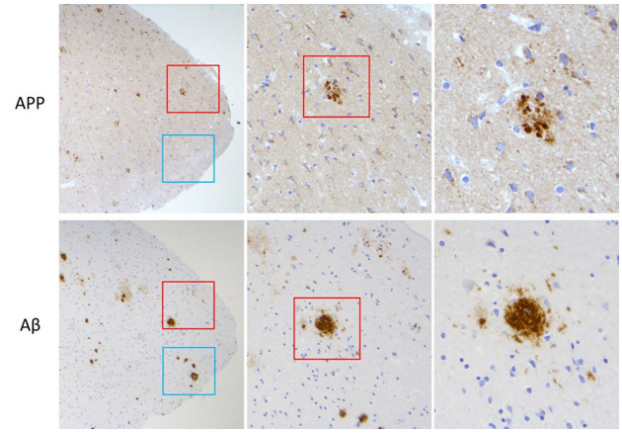


**Figure 2:** Comparison and correlation of APP plaques with Aβ staining in human AD brain. There is evidence of areas with APP and Aβ co-aggregation (red box) and lack of correlation (blue box). Samples from 74 year old male with clinical history of dementia.

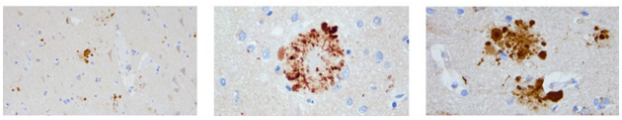


**Figure 3:** Samples of APP aggregate morphology from human brain samples. APP aggregates range from point-like, punctate structures (left) to more diffuse (middle) and "plaque"-like structures (right). Aggregates appear to be extracellular and mimic dystrophic neurites, although this has yet to be confirmed. Punctate APP structures remain poorly defined in the literature in terms of location and function. Samples include right frontal cortex (66 year old M – Lewy body dementia, 20x), right superior temporal cortex (52 year old female - Frontotemporal dementia, 40x), and right frontal cortex (77 year old male – AD, 40x).

mutations). Mutations affecting APP (duplication or V717I mutation) increased tau levels, while mutations in PSEN1 did not [107, 108]. The V717I and PSEN1 mutated neurons also showed similar levels of extracellular Aβ, suggesting an Aβ-independent mechanism of tau accumulation [108]. Blockade of γ-secretase in these neurons increased tau while the opposite was true for β-secretase inhibition (both treatments reduced Aβ, as expected). These changes appeared to correlate with production of the C-terminal fragment APP-C83/99, which has previously been shown to accumulate intracellularly in transgenic mouse models [109]. Additionally, C99 is increased in brains of autosomal-dominant AD patients and associated with endosomal dysfunction and increased expression of glycogen synthase kinase-3 beta, a crucial kinase in pathogenic tau phosphorylation [102, 110, 111]. Furthermore, iPSC-derived neurons from Down syndrome patients, who carry a triplication of chromosome 21 (and thus the APP locus), also demonstrate tau pathology and Aβ formation [112, 113]. This suggests that APP expression and potentially C99 in particular are responsible for modulating tau pathology. Contrastingly, when the extra APP gene was knocked out in Down syndrome iPSCs there was no significant reduction in hyperphosphorylated tau; similarly, APP overexpression in euploid human embryonic stem cells increased total tau but not T212-phosphorlyation, which is associated with AD pathology [114]. While these results are preliminary and conflicting, they constitute a small sample size and suggest a distinct role of APP processing independent of Aβ in tau formation. Recently, somatic APP mutations have been identified in human sporadic AD neurons. These mutations appear to result from reverse-transcription of RNA that is incorporated into the DNA, resulting in increased APP gene copy number and even mutations associated with familial AD [115, 116]. These genetic variants may contribute to increased Aβ production or represent novel toxic products that accumulate in susceptible neurons, although the results have to be validated in a larger sample with more robust controls. Given these examples of APP dysregulation and dysfunction early in disease, it is likely a contributor to AD pathogenesis that eventually results in Aβ production/aggregation.

## Non-Traditional Role of APP in Chronic Traumatic Encephalopathy

While the role of APP is well-recognized in AD, it is unclear as to how it may be involved in other neurodegenerative diseases. One particularly interesting example includes chronic traumatic encephalopathy (CTE). This pathology is characterized in part by tau-immunoreactive neurofibrillary tangles found preferentially in superficial cortical layers and tau-reactive astrocytes [117, 118]. CTE is clinically characterized by attention, concentration, and memory deficits which can progress to overt dementia and slowed muscular movement [117, 119]. It often develops following multiple cases of traumatic/acute brain injury, generally seen in professional athletes [118, 120].

One of the similarities between CTE and AD risk is the link to previous traumatic brain history; multiple studies have identified a correlation between history of acute head injury and later development of AD, with the general consensus that head trauma (even a single occurrence) contributes to AD risk [121-126]. There is also evidence that as soon as a few years following traumatic brain injury, patients can demonstrate Aβ, APP and tau pathology in the brain [127-130]. This is likely due to axonal injury and impaired axonal transport, resulting in APP accumulation and processing. This processing involves increased formation of beta-C terminal fragments, which was shown in a rodent model of traumatic brain injury [131]. In this model there was also a loss of full length tau and increase in the 22 kD form, which has been implicated in AD [131-133]. This suggests that brain injury could favor processing of APP and tau towards a more pathologic form. There is also neuropathological correlation between AD and CTE in that many CTE cases demonstrate accumulations related to AD and Lewy Body Disease. In particular, studies have reported between 50-100% of their examined CTE cases contain Aβ deposits in the form of both diffuse and neuritic plaques [120, 130, 134]. These findings suggest that APP and tau metabolic changes occur in both the acute and chronic setting following a TBI and correlate with CTE pathology, although a direct pathological role has yet to be identified.

## The Amyloid Precursor Hypothesis

Given the above evidence, we present a novel interpretation of the Aβ hypothesis whereby APP plays a more prominent role in pathology, independent of Aβ production. In this model, Aβ is a contributory but not central influence in AD pathogenesis, which is supported by the following: individuals with significant Aβ burden show little cognitive decline [81-84, 135]; clinical trials targeting Aβ have failed to produce significant results [136]; multiple additional pathways exist that cleave APP into toxic products (Table 1); and altering APP metabolism without increasing Aβ levels influences tau pathology [107, 108]. Interestingly, many of the familial AD mutations affecting APP and the γ-secretase complex lead to a partial loss of cleavage and function [137-140]. Furthermore, in TgCRND8 AD mice, treatment with a γ-secretase inhibitor failed to rescue all memory deficits as compared to β-secretase [141]. This was attributed to an Aβ-independent mechanism most likely involving C99, the C-terminal fragment produced following initial APP cleavage by β-secretase. The C99 component has also been related to tau levels in several studies, albeit indirectly. In the aforementioned study by Moore et al, γ-secretase inhibition *in vitro* increased tau levels while β-secretase inhibition had the opposite effect [108]. Theoretically, γ-secretase inhibition would prevent processing of C99 following initial β-secretase cleavage. These results were also repeated using induced pluripotent stem cells of familial and sporadic AD patients [142]. Together, these inconsistencies point to an alternative contributor in pathogenesis. APP thus emerges as a central player that produces a key marker of AD (Aβ) but may significantly contribute to other aspects of pathology in an Aβ-independent fashion.

We thus hypothesize that APP metabolism and manipulation,

prior to Aβ formation, contributes directly to AD pathogenesis and influences tau pathology. We recognize that AD is a multi-factorial and complex disease process that is unlikely to have a single causative factor. APP is near the centre of a complex web of interactions involving inflammatory signaling (including microglia and other immune cells) [143], impaired waste product clearance [144], and cellular senescence [145, 146]. However, APP plays an underappreciated role in pathogenesis, particularly tau pathology that deserves further investigation. These studies may offer novel therapeutic targets and greater understanding of this complex and heterogenous disease.

## Synopsis

The amyloid-beta hypothesis is giving way to a more holistic paradigm of Alzheimer Disease pathogenesis with a greater focus on components such as the amyloid precursor protein. Current research has identified novel roles for this protein in normal physiology and disease, independent of amyloid-beta formation. Furthermore, there is evidence supporting a previously underappreciated interaction between amyloid precursor protein and tau that may prove to be crucial to disease pathogenesis. These findings highlight a unique role for amyloid precursor protein in various neurodegenerative diseases and the potential for new therapeutic avenues.

## Conflict of Interest

The authors have no conflicts of interest to declare.

## Acknowledgements

Michael G. DeGroote School of Medicine for providing funding in the form of the MacRES scholarship.

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