7

N-Terminally Truncated Aβ Peptide Variants in Alzheimer's Disease

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Abstract: The accumulation and aggregation of amyloid-β (Aβ) peptides in the brain is believed to be the initial trigger in the molecular pathology of Alzheimer's disease (AD). In addition to the widely studied full-length Aβ peptides (mainly $Aβ_{1–40}$ and $Aβ_{1–42}$), a variety of amino-terminally truncated (N-truncated) peptides, such as $Aβ_{pE3-x}$ and $Aβ_{4-x}$, have been detected in high abundance in autopsy samples from sporadic and familial AD patients. N-truncated Aβ species adopt specific physicochemical properties resulting in a higher aggregation propensity and increased peptide stability, which likely account for their neurotoxic potential. The presence of N-truncated Aβ peptides in transgenic mouse models of AD and the selective overexpression of specific N-truncated variants in the murine brain have facilitated their investigation in relevant in vivo settings. In this chapter, we address the pathological relevance of N-truncated Aβ peptide species and summarize the current knowledge about the enzymatic activities that might be involved in their generation.

Keywords: ADAMTS4; Alzheimer's disease; amyloid; N-truncation; protease

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INTRODUCTION

The deposition of extracellular plaques consisting of amyloid-β (Aβ) peptides in the brain parenchyma is one of the neuropathological hallmarks of Alzheimer's disease (AD). Although these deposits have also been found in non-demented control individuals, they are believed to play an important role in the disease process, and their presence and abundance is an obligatory criterion for a diagnosis of AD. Full-length Aβ peptides composed of 40 ($Aβ_{1–40}$) or 42 ($Aβ_{1–42}$) amino acids constitute the main components of extracellular amyloid plaques, together with other proteins such as ubiquitin and different proteoglycans. These peptides are generated by sequential proteolytic cleavage of the amyloid precursor protein (APP), a large type-I transmembrane protein that in rare families was found to carry mutations causative of inherited cases of AD. After an initial cleavage by either α- or β-secretase, which facilitates shedding of the APP ectodomain, the remaining membrane-bound β- or α-C-terminal fragments (CTFs) are cleaved by γ-secretase within their transmembrane domains. In the latter case, a small peptide fragment named p3 is released, while the cleavage of β-CTFs results in the generation of Aβ peptides (1) (Figure 1).

The analysis of brain samples from non-demented control cases, pathological aging (which is being regarded as a prodromal phase of AD), and AD revealed that, apart from full-length $Aβ_{1–40}$ and $Aβ_{1–42}$, N-truncated $Aβ_{x-42}$ species were the most abundant in AD with considerable overlap in pathological aging samples (2). This is interesting from a pathological point of view as full-length Aβ peptides are normal metabolites generated under physiological conditions. The exact physiological function of these peptides remains unresolved; however, it has been hypothesized that modulation of endogenous Aβ production might play an important role in the regulation of neuronal activity via a feedback loop mechanism (3). Other possible physiological functions include promoting recovery from traumatic brain injury, sealing leaks in the blood–brain barrier, or antimicrobial activities (4). While full-length Aβ peptides starting with an aspartic acid (Asp) residue at position 1 of the Aβ sequence are generated by an enzymatic activity called β-site APP cleaving enzyme 1 (BACE1) (5, 6), much less is known about the proteases responsible for the production of N-truncated Aβ peptides.

Aβ peptides with varying N-termini were described more than 30 years ago. In 1984, the identification of full-length Aβ peptides starting with an Asp residue in position 1 purified from cerebrovascular amyloid deposits was reported (7). The following year, N-terminal sequencing of Aβ peptides purified from amyloid plaque cores from AD cases demonstrated the presence of peptides starting with phenylalanine (Phe) in position four ($Aβ_{4-x}$), as well as with serine (Ser) or glycine (Gly) in position eight ($Aβ_{8-x}$) or nine ($Aβ_{9-x}$) (8, 9). By means of immunohistochemistry, N-truncated Aβ species with post-translational modifications such as pyroglutamylation at position 3 ($Aβ_{pE3-x}$) and 11($Aβ_{pE11-x}$) were subsequently described in human AD brains (10, 11). The loss of charged amino acids at the N-terminus changes the biophysical properties of the Aβ peptides, thus influencing their aggregation propensity and toxicity. As a consequence, efforts to understand the relevance of N-truncated Aβ species in the pathogenesis of AD, as well as the mechanisms responsible for their generation, have recently increased.

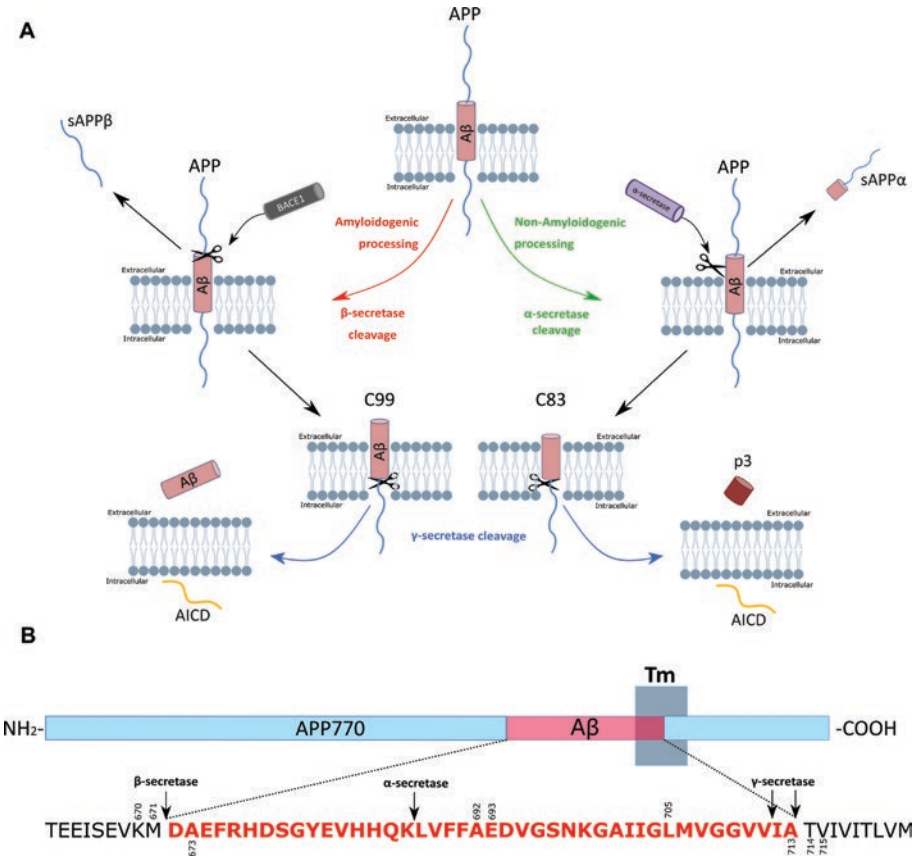

Figure 1 **APP processing pathways. A**) The non-amyloidogenic processing pathway (depicted on the right) is initiated through cleavage by α-secretase, which cleaves within the Aβ domain and generates the soluble ectodomain sAPPα. Subsequent cleavage of the membrane-bound C-terminal APP fragment C83 by the γ-secretase complex releases the soluble fragments p3 and the APP intracellular domain (AICD). Amyloidogenic APP processing (left panel) is initiated by β-secretase cleavage with the liberation of the soluble sAPPβ fragment. The remaining C-terminal fragment C99 is then cleaved by γ-secretase generating Aβ peptides as well as AICD. **B**) APP is a large transmembrane protein containing up to 770 amino acids. The Aβ peptide sequence (in red) starts within the ectodomain and ends within the transmembrane (TM) domain.

HETEROGENEITY OF N-TRUNCATED Aβ SPECIES IN AD BRAIN

Several studies employing mass spectrometry (MS) that intended to analyze the full spectrum of Aβ peptides in postmortem brain samples of AD patients have been published. In the earliest of these studies, purified amyloid core and cerebrovascular amyloid peptides were sequenced using matrix-assisted laser-desorption-time-of-flight (MALDI-TOF) mass spectrometry. While the amino acid composition of cerebrovascular Aβ peptides consisted mainly of species

starting with residues 1 or 2, the preparations from amyloid cores were more heterogeneous, corresponding to peptides beginning with every residue between Asp-1 and Glu-11(Figure 2), with major signals for peptides starting with Phe-4, Ser-8, and Glu-11 (12). In good agreement, using surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) mass spectrometry, $A\beta_{4\text{-}42}$ was also identified as the major N-truncated species in postmortem brain samples from aged controls, patients with vascular dementia, and AD patients (13). This suggested that N-truncated species account for a substantial proportion of total Aβ in the aged human brain, a finding that was corroborated in subsequent studies. The entire spectrum of Aβ peptides ranging from $A\beta_{1\text{-}x}$ to $A\beta_{pE11\text{-}x}$was detected in frontal cortex samples of a sporadic AD case and of an individual affected by the FAD-associated presenilin (*PSEN1)* V261I mutation. This mutation is associated with the deposition of the so-called cotton wool plaques, which are lesions lacking a central amyloid core (14). By investigating non-demented individuals with incipient amyloid pathology as well as AD patients, it was further demonstrated that initial insoluble Aβ aggregates are largely composed of N-truncated Aβ42 variants such as peptides starting at positions 4-, 5-, 8-, or 9–42 (15). Portelius et al. also studied the Aβ isoform pattern in the hippocampus, cortex, and cerebellum of non-demented controls, sporadic AD cases, and patients suffering from familial AD (FAD). In all groups, $A\beta_{1\text{–}42}$, $A\beta_{1\text{–}40}$, $A\beta_{pE3\text{–}42}$, and $A\beta_{4\text{–}42}$ were identified as the dominant isoforms (16), which is in good agreement with the most recent studies from other investigators (2, 17, 18).

N-TERMINALLY TRUNCATED Aβ SPECIES IN TRANSGENIC MOUSE MODELS OF AD

Transgenic mouse models overexpressing mutant forms of human APP, either alone or in combination with mutant forms of *PSEN1* or *PSEN2*, are valuable and

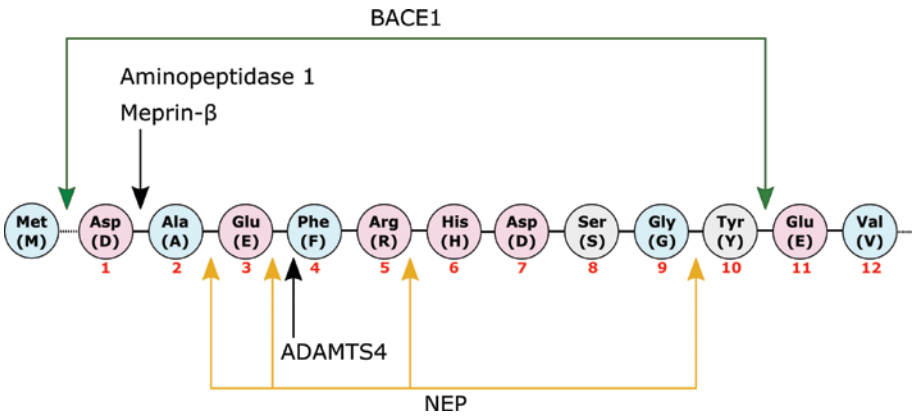

Figure 2 Sequence of the Aβ N-terminus with indicated cleavage sites and enzymes involved in the generation of N-truncated Aβ species. Amino acids (AA) are color-coded according to their properties (red: charged AA; grey: uncharged AA; blue: nonpolar hydrophobic AA).

widely used model systems to study AD-associated pathological alterations such as extracellular amyloid deposition, inflammatory responses, and cognitive deficits (19–21). The analysis of Aβ peptide species in brain samples using mass spectrometry revealed that most transgenic AD mouse models only partially reflect the Aβ spectrum in human sporadic AD. While the overall heterogeneity of N-terminal truncated Aβ species could be reproduced in mouse models such as APP/PS1KI (22) or 5XFAD (23, 24), the ratio of full-length Aβ peptides to N-truncated variants is much different in human brain samples. While N-truncated variants such as $Aβ_{pE3-x}$ or $Aβ_{4-x}$ might be present in comparable quantities compared to full-length $Aβ_{1–40}$ or $Aβ_{1–42}$ species in human samples (16), full-length peptides comprise by far the majority of all Aβ peptides in transgenic AD models (23, 25, 26). This is likely explained by the fact that most of these models (e.g., Tg2576 (27), APP23 (28), APP/PS1KI (22), 5XFAD (29), or APPswe/PSEN1dE9 (30)) utilize the Swedish APP mutation. Cell lines transfected with the Swedish APP670/671 mutation have been shown to release three to six times more Aβ peptides than wild-type cells (31, 32). Due to the location of the double mutation in the immediate vicinity of the β-secretase cleavage site (Figure 1), the Swedish mutation increases the affinity of the substrate APP for BACE1, thus favoring the generation of full-length Aβ peptides starting with Asp in position 1 (33).

Using two-dimensional gel electrophoresis with subsequent mass spectrometry analysis, a variety of N-truncated Aβ species have been detected in APP/PS1KI mice. While full-length $Aβ_{1–42}$ peptides were already detectable in young mice at 2.5 months of age, other Aβ variants such as $Aβ_{2/3–42}$, $Aβ_{pE3–42}$, and $Aβ_{4/5–42}$ became apparent only at later time points (22). Mass spectrometry analyses have also supported that N-truncated species represent only a small percentage of the total Aβ peptide amount in mouse models such as 5XFAD or APP23, although variable ionization efficiencies for the different Aβ species might contribute to a distorted image of the Aβ peptide composition in both mouse and human brains (23, 34). In conclusion, N-truncated Aβ species are substantially underrepresented in transgenic mouse models compared to human AD brain samples (34, 35).

MAJOR N-TERMINAL TRUNCATED Aβ SPECIES DETECTED IN HUMAN BRAIN

As pointed out above, a huge variety of different N-terminal truncated Aβ species has been identified by either MS or immunohistochemical staining methods in brain samples from human AD patients. In this section, we discuss the current knowledge on the most important variants in more detail.

$Aβ_{2-x}$

In AD patients, a consistent elevation of Aβ peptides lacking the N-terminal Asp residue have been observed in the detergent-soluble pool of brain extracts, as well as in cerebrospinal fluid (CSF) samples (36). Using SELDI-MS, several Aβ peptides including those starting with Ala-2 were found in extractions from senile plaques (13). Immunohistochemical analysis of postmortem brain samples using

an $A\beta_{2\text{-}x}$-specific polyclonal antibody confirmed the presence of $A\beta_{2\text{-}x}$ peptides in both parenchymal and vascular deposits of sporadic AD cases as well as transgenic mouse models such as APP/PS1KI or 5XFAD (37). As the sequence of full-length Aβ starts with an Asp residue in position 1, it has been suggested that proteolysis of $A\beta_{1\text{-}x}$ peptides by the exopeptidase aminopeptidase A, which releases Glu and Asp residues from the N-termini of proteins, could result in the generation of $A\beta_{2\text{-}x}$ species (38). However, the evidence in this study was limited to showing that Western blot immunoreactivity with an $A\beta_{1\text{-}x}$-specific antibody was reduced after the co-incubation of purified aminopeptidase A with recombinant full-length $A\beta_{1–40}$ peptides (Table 1). The identity of specific degradation products and, in particular, the generation of $A\beta_{2\text{-}x}$ species, was not confirmed by mass spectrometry or other methodology (38). In contrast, it has been convincingly demonstrated in cell-free and cell-based assays that cleavage of APP or Aβ by the metalloprotease meprin-β can result in the generation of $A\beta_{2\text{-}x}$ species (39, 40). In both HEK293T and CHO cells, co-expression of human APP and meprin-β facilitated the secretion of $A\beta_{2–40}$ peptides, whose identity was confirmed by mass spectrometry, and this was blocked by treatment with a γ-secretase but not a β-secretase inhibitor, indicating that $A\beta_{2–40}$ peptides were produced through a BACE1-independent mechanism. Later, these results were partially confirmed by another group (41). Still missing is in vivo proof that meprin-β is responsible for the brain production of $A\beta_{2\text{-}x}$ peptides in AD mouse models. However, this experiment is complicated by the fact that meprin-β does not generate $A\beta_{2\text{-}x}$ peptides with Swedish mutant APP as a substrate, which excludes most of the commonly used APP-transgenic strains as in vivo model systems (40).

$A\beta_{pE3\text{-}x}$

Pyroglutamate-modified $A\beta_{pE3\text{-}x}$ represents a major Aβ species identified in human AD brains (16, 50). In 1995, Saido et al. reported the identification of these

TABLE 1 **List of proteases involved in the generation of N-truncated Aβ species**

Protease	Levels/activity in human AD brain versus control	Cleavage site	Potential Aβ peptides	References
BACE1	Increased (42)	Met(-1) ↓ Asp(1) Tyr(10) ↓ Glu(11)	$A\beta_{1\text{-}x}$ $A\beta_{11\text{-}x}$, $A\beta_{pE11\text{-}x}$	(6)
Aminopeptidase A	Reduced (43)	Asp(1) ↓ Ala(2)	$A\beta_{2\text{-}x}$	(38)
Meprin-β	Unknown	Asp(1) ↓ Ala(2)	$A\beta_{2\text{-}x}$	(39, 40)
Neprilysin (NEP)	Increased (44) Reduced activity (45)	Asp(2) ↓ Ala(3) Ala(3) ↓ Phe(4) Arg(5) ↓ His(6) Gly(9) ↓ Tyr(10)	$A\beta_{3\text{-}x}$, $A\beta_{pE3\text{-}x}$ $A\beta_{4\text{-}x}$ $A\beta_{6\text{-}x}$ $A\beta_{10\text{-}x}$	(46, 47) (48, 49)
ADAMTS4	Unknown	Ala(3) ↓ Phe(4)	$A\beta_{4\text{-}x}$	(24)

post-translationally modified peptides in which the glutamate at position three becomes converted to pyroglutamate through intramolecular dehydration (51). This cyclization alters the physicochemical properties of Aβ and results in increased hydrophobicity due to the loss of a negative charge, faster aggregation kinetics compared to full-length Aβ peptides in in vitro assays (52–54), and increased insolubility and stability (55). Importantly, higher abundance of these peptides in AD as compared to age-matched non-demented control patients has been demonstrated (56–58). With regard to their toxic properties, increased neurotoxicity compared to full-length Aβ peptides (59) has been reported; however, some studies found full-length and $Aβ_{pE3-x}$ peptides to be equally toxic (60, 61), while others suggested that $Aβ/Aβ_{pE}$ hetero-oligomers constitute the main neurotoxic Aβ fraction (62). Interestingly, related properties have also been reported for pyroglutamylated ABri and ADan peptides, representing the major peptide species accumulating in the neurodegenerative disorders familial British dementia and familial Danish dementia (63, 64).

The formation of $Aβ_{pE3-x}$ peptides appears to be at least a two-step process, with removal of the first two amino acid residues from full-length Aβ followed by cyclization. Recently, it has been suggested that meprin-β might not only generate $Aβ_{2-x}$ but also $Aβ_{3-x}$ peptides as substrates for cyclization (41). However, $Aβ_{3-x}$ peptides were not detected in an earlier study by mass spectrometry (39), and whether genetic deletion of meprin-β would reduce $Aβ_{pE3-x}$ peptide formation in vivo is unknown. In contrast, solid evidence supports that glutaminyl cyclase (QC) is at least one of the enzymes capable of catalyzing the second step of $Aβ_{pE3-x}$ formation (65). Treatment using an orally available QC inhibitor resulted in a reduction of the $Aβ_{pE3–42}$ burden in transgenic mouse models of AD (66). The same was also seen in 5XFAD mice on a QC knock-out background and was accompanied by a rescue of behavioral deficits (67). The observation of a significant age-dependent increase of the $Aβ_{pE3-x}$ parenchymal plaque burden at the expense of $Aβ_{1-x}$ full-length peptides suggested that $Aβ_{pE3-x}$ formation might occur late in the process of amyloidosis and could involve the remodeling of existing extracellular amyloid deposits (68). On the other hand, the presence of $Aβ_{pE3-x}$ peptides has been also described within neurons both in mouse models (22, 69) and human AD samples (53, 70), raising the question of whether the localization is important for toxicity. In order to address such questions, transgenic mouse models have been developed with constructs that only encode the $Aβ_{3-x}$ peptide, with a glutamate to glutamine substitution at the initial position to facilitate cyclization (71–73). This construct is expressed under the control of the murine neuron-specific Thy1-promotor and contains the thyrothropin-releasing hormone (TRH) signal peptide sequence to ensure liberation of the peptide preferentially in the secretory pathway (74). In contrast to other models, these mice do not express human full-length APP or any FAD-associated mutations, but impress with a rapid onset of behavioral deficits, neuron loss, and microgliosis (71, 72).

$Aβ_{4-x}$

$Aβ_{4–42}$ was one of the first Aβ peptide species that was detected in the amyloid plaque cores of human AD brains (9). More recently, novel $Aβ_{4-x}$ specific antibodies have been described, and the localization of $Aβ_{4-x}$ to amyloid plaque cores has been confirmed in immunohistochemical studies in both human AD and

transgenic AD mouse models (75, 76). In addition, $A\beta_{4\text{-}x}$ peptides were also found within blood vessels in the majority of the analyzed AD cases (76). Similar to $A\beta_{pE3\text{-}x}$ peptides, $A\beta_{4–42}$ peptides lacking another charged amino acid residue have also been described to quickly aggregate into soluble oligomers and fibrillar, high-molecular weight aggregates (61, 75, 76). Quantitatively, $A\beta_{4–42}$ peptides seem to be among the most abundant Aβ species in human AD brain with equal or even higher amounts compared to $A\beta_{1–42}$. It should be noted again, however, that in studies using mass spectrometry to assess Aβ peptide patterns, the ratios between the respective peptide variants cannot be regarded as a direct reflection of their abundance (16, 77). With regard to their neurotoxicity, $A\beta_{4–42}$ and $A\beta_{4–40}$ demonstrated equal toxicity as $A\beta_{1–42}$ or $A\beta_{pE3–42}$ using in vitro assays with primary neuronal cultures. This was also observed in an in vivo setting in which freshly prepared Aβ peptides were applied by intraventricular injection followed by an analysis of working memory using a Y-maze task after 5 days (61).

The metalloprotease neprilysin (NEP) has been proposed as a candidate enzyme responsible for the generation of $A\beta_{4\text{-}x}$ peptides by cleaving between Glu-3 and Phe-4 among other sites, with full-length $A\beta_{1\text{-}x}$ peptides acting as the immediate substrate. This has been shown by high-performance liquid chromatography analysis yielding several product peaks after incubation of $A\beta_{1–40}$ with either recombinant soluble NEP produced in Sf9 cells or NEP purified from rabbit kidney cortex (46). More recent studies using synthetic Aβ peptides and recombinant human NEP confirmed the generation of Aβ peptide fragments starting with Phe-4 (such as $A\beta_{4–9}$ or $A\beta_{4–16}$ but also the existence of several other cleavage sites, at least under the given in vitro conditions (48, 49). Therefore, it is currently unclear whether NEP might contribute to the generation of longer Aβ peptides such as $A\beta_{4–40}$ and $A\beta_{4–42}$. However, we regard this possibility as unlikely as in vivo studies have demonstrated that the rate-limiting step in the proteolysis of Aβ by NEP is cleavage of the Gly-9–Tyr-10 bond, which would rule out the generation of full-length $A\beta_{4–40}$ and $A\beta_{4–42}$ peptides (78).

Most recently, it was shown that APP contains a cleavage site for the metalloprotease ADAMTS4 (a disintegrin-like and metalloprotease with thrombospondin type 1 motif) between Glu-3 and Phe-4 of the Aβ peptide sequence (24). ADAMTS proteases constitute a family of secreted Zn^{2+}-metalloproteases that degrade or modify major components of the extracellular matrix (79). ADAMTS4 participates in the proteolytic degradation of proteoglycans like aggrecan, brevican, and versican (80). Aggrecan is a hyaluronan-binding proteoglycan, which is present in large amounts in the articular cartilage. In an important pathological process leading to osteoarthritis and rheumatoid arthritis, aggrecan is degraded by ADAMTS4 and the homologous family member ADAMTS5, leading to the exposure and subsequent degradation of collagen fibrils by collagenases (81). Co-expression of ADAMTS4 and APP in HEK293 cells resulted in the secretion of $A\beta_{4–40}$ peptides as measured by mass spectrometry and ELISA, while several species of $A\beta_{1\text{-}x}$ peptides were not affected (24). $A\beta_{4–40}$ secretion was not blocked by treatment of the cells with a potent β-secretase inhibitor indicating that $A\beta_{4\text{-}x}$ peptides were generated in a BACE1-independent fashion. IHC analysis of ADAMTS4 reporter mice showed that ADAMTS4 was exclusively expressed in oligodendrocytes in the adult murine brain. Consistently, the culture of murine oligodendrocytes demonstrated that these primary cells secrete $A\beta_{4–40}$ peptides among a spectrum of other Aβ species very similar to established cell lines. However, $A\beta_{4–40}$ peptides were

undetectable in primary oligodendrocytes derived from ADAMTS4 knockout (KO) mice, providing genetic proof that ADAMTS4 is responsible for $A\beta_{4–40}$ peptide generation in this cell type. In vivo, the crossing of 5XFAD mice to ADAMTS4 knockout mice reduced $A\beta_{4–40}$ levels by 50%, but the overall amyloid plaque load and the distribution of $A\beta_{4\text{-}x}$ peptides in amyloid plaque cores appeared to be unchanged, clearly suggesting that other mechanisms for $A\beta_{4\text{-}x}$ generation beside ADAMTS4 must exist. Compellingly, abundant $A\beta_{4\text{-}x}$ immunoreactivity was observed in white matter structures of 5XFAD mice, and this signal was entirely abolished in the ADAMTS4 knockout background (24). This could be of pathological relevance as numerous neuropathological, biochemical, and imaging studies have reported white matter abnormalities and oligodendrocyte dysfunction in AD patients (82, 83). However, further studies are required to define a potential detrimental role of $A\beta_{4\text{-}x}$ peptides in white matter structures. In any case, the recent link of ADAMTS4 to AD risk as well as single-cell transcriptomic data supporting that many oligodendroglia-specific and myelination-associated genes are dysregulated in human AD brains should provide new urgency to consider the role of oligodendrocytes in AD (84, 85).

As a tool to investigate the in vivo role of $A\beta_{4\text{-}x}$ peptides, a transgenic mouse model has been generated that only expresses $A\beta_{4–42}$ peptides under the control of the murine Thy1-promotor. These mice develop age-dependent behavioral deficits with spatial or working memory impairments, which are detectable in paradigms such as Morris water maze or novel object recognition task, as well as motor deficits. These mice do not develop amyloid plaque pathology but show a robust hippocampal CA1 neuron loss correlating with the transgene expression pattern in a gene-dose dependent manner (61, 86). Interestingly, altered basal excitatory synaptic transmission with $A\beta_{4–42}$-dependent neuronal hyperexcitability is already obvious in young Tg4–42 mice preceding neuron loss and behavioral deficits (87).

$A\beta_{5\text{-}x}$

Aβ peptides starting with an Arg residue at position 5 have been detected in brains of transgenic mice such as APP/PS1KI (22) or 5XFAD (23), as well as in human AD brains (15–17) by mass spectrometry. Conditions of BACE1 inhibition resulted in strongly increased levels of $A\beta_{5\text{-}x}$ species in cellular models (88–90). This clearly suggests that $A\beta_{5\text{-}x}$ peptides are produced through a BACE1-independent pathway, with some evidence supporting α-secretase-like proteases (e.g., ADAM family proteases such as TACE or ADAM10) as potential candidate enzymes (88).

In vivo studies with several BACE1 inhibitors in beagle dogs confirmed the absolute signal reduction of all Aβ isoforms in the CSF except for $A\beta_{5–40}$ peptides, and an analysis of relative levels demonstrated a clear increase of $A\beta_{5–40}$ (90). This was further corroborated in a placebo-controlled study in healthy human subjects in which dose-dependent increases in $A\beta_{5\text{-}x}$ levels were measured in the CSF upon treatment with the BACE1 inhibitor LY2811376 (91). Immunohistochemical analyses using $A\beta_{5\text{-}x}$ selective antibodies confirmed the presence of $A\beta_{5\text{-}x}$ peptides in brain tissues samples from sporadic AD patients showing immunoreactivity primarily in vascular deposits (88, 92). In cases from individuals harboring FAD-associated *APP* or *PSEN1* mutations, both vascular

and parenchymal deposits were detected, while in mouse models such as 5XFAD, APP/PS1KI, or 3xTg $A\beta_{5\text{-}x}$, immunoreactivity was confined to extracellular plaques (92).

$A\beta_{11\text{-}x}$/$A\beta_{pE11\text{-}x}$

In addition to the cleavage site between methionine and aspartate in position 1 (Asp-1) generating β-CTFs, BACE1 has also been shown to cleave APP between tyrosine and glutamate in position 11 (Glu-11) of the Aβ sequence resulting in N-truncated $A\beta_{11\text{-}x}$ species (β′-cleavage) (6). There is evidence that the BACE1 cleavage preference depends on the intracellular localization, with β′-cleavage being favored in the trans-Golgi network (93). $A\beta_{11\text{-}x}$ peptides have been detected in brains from AD and Down's syndrome patients (94) and have been shown to accumulate within neurons in cellular models upon BACE1 overexpression (95). Similar to Glu-3, the free Glu residue in position 11 can also undergo cyclization and modification to an N-terminal pyroglutamate ($A\beta_{pE11\text{-}x}$). In contrast to $A\beta_{pE3\text{–}42}$, which is mainly confined to mature plaque cores in AD patients, unmodified $A\beta_{11\text{–}40}$ and $A\beta_{pE11\text{–}40}$ peptides have been detected in the vasculature using selective antibodies (95). Within amyloid plaques cores, $A\beta_{pE11\text{-}x}$ has been found to co-localize with full-length Aβ peptides but also with $A\beta_{pE3\text{-}x}$ (96).

CONCLUSION

There is substantial evidence that N-truncated Aβ species, in addition to the extensively studied full-length Aβ peptides, might play an important role in the molecular pathology of AD. In recent years, new candidate proteases and nonneuronal cell types have been linked to the generation of N-truncated Aβ species. Novel antibodies specific for some N-truncated Aβ peptides have been developed, and this should allow the development of quantitative detection assays to better define their abundance in relation to full-length Aβ peptides. To advance the functional analysis of N-truncated Aβ peptides, novel animal models might be needed as N-truncated Aβ species are underrepresented in the available AD models. These efforts should improve our understanding of the pathological role of N-truncated Aβ peptides. They could provide novel insights into currently unexplained aspects of AD pathology, and they might be crucial to develop novel therapeutic approaches.

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