Secretases in Alzheimer's disease: Novel insights into proteolysis of APP and TREM2

Stefan F. Lichtenthaler[1,2,3], Sarah K. Tschirner[1,2] and Harald Steiner[1,4]

[1]German Center for Neurodegenerative Diseases (DZNE), Munich, Germany;
[2]Neuroproteomics, School of Medicine, Klinikum rechts der Isar, Technical University of Munich, 81675 Munich, Germany
[3]Munich Cluster for Systems Neurology (SyNergy), Munich, Germany
[4]Biomedical Center (BMC), Division of Metabolic Biochemistry, Faculty of Medicine, LMU Munich

To whom correspondence should be addressed:
stefan.lichtenthaler@dzne.de or harald.steiner@med.uni-muenchen.de
Postal address: Feodor-Lynen-Street 17, 81377 Munich, Germany, +49.89.4400.46426 (SFL) or +49.89.4400.46535 (HS), Fax: +49.89.4400.46408

Highlights

- Sfrp1, RECK, Atxn1 and Nrf2 control ADAM10 and BACE1 activity and are linked to AD
- New familial Alzheimer Uppsala mutation shifts APP cleavage from α- to β-secretase
- Structure and pharmacology studies support drug development of γ-secretase modulators
- TREM2-agonistic antibodies show potential for reducing brain amyloidosis in mice

Short Title: Secretases in Alzheimer's disease

Abstract

Secretases are a group of proteases that are major drug targets considered for prevention and treatment of Alzheimer's disease (AD). Secretases do not only process the AD-linked neuronal amyloid precursor protein (APP), but also the triggering receptor expressed on myeloid cells 2 (TREM2), thereby controlling microglial functions. This review highlights selected, recent discoveries for the α-secretases ADAM10 and ADAM17, the β-secretase BACE1 and γ-secretase and their link to AD. New genetic evidence strengthens the role for α-secretases in AD through cleavage of APP and TREM2. Novel proteins were linked to AD which control α- and β-secretase activity through transcriptional and post-translational mechanisms. Finally, new opportunities, but also challenges are discussed for pharmacologically targeting β- and γ-secretase cleavage of APP and α-secretase cleavage of TREM2, with the aim to prevent or treat AD.

Keywords: Alzheimer's disease, APP, RECK, secretases, Sfrp1, TREM2

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease and affects more than 50 million patients worldwide. The onset of clinical AD symptoms is preceded by an approximately 20 year-long asymptomatic disease pathogenesis that comprises a complex series of molecular and cellular events, referred to as the amyloid cascade hypothesis [1] (Fig. 1) which is based on genetic and epidemiological evidence [2]. The cascade starts with the formation of small, oligomeric aggregates of the hydrophobic amyloid β (Aβ) peptide, which proteolytically derives from the amyloid precursor protein (APP). Because Aβ is mostly produced and released from neurons, AD pathogenesis was long seen from a neuron-centric perspective. However, this view changed in the recent past with the discovery that numerous genetic AD risk factors for late onset AD (LOAD), such as apolipoprotein E (ApoE) and triggering receptor expressed on myeloid cells 2 (TREM2), are strongly or even exclusively expressed in microglia [3]. This observation led to the discovery of beneficial, but also detrimental functions for microglia in AD pathogenesis. As immune cells of the brain, microglia can adapt to changing environments by modulating gene expression from homeostatic to protective disease-associated states [4,5]. Microglia can phagocytose and, thus, reduce abundance of Aβ, but also contribute to Aβ aggregation and excessive neuroinflammation (Fig. 1). TREM2 has emerged as a major player governing beneficial microglial defense responses to pathological Aβ deposition including microglial proliferation, chemotaxis, motility and survival in addition to Aβ phagocytosis [3]. As a defense mechanism to AD pathology, TREM2 appears to sense Aβ aggregates early on and, together with ApoE, with which TREM2 physically interacts, triggers a probably beneficial compaction of amyloid plaques to reduce neuritic damage as well as to prevent the release of toxic oligomeric Aβ forms from the outer plaque halos by providing a microglial barrier function [3].

Proteolysis controls abundance or activity of most proteins. In AD, proteolysis has been studied most intensively for APP as it regulates generation and abundance of Aβ [1]. Similar proteolytic processing pathways have been identified more recently for TREM2 and control the phagocytic activity of microglia, which is one mechanism to degrade Aβ [3]. Pharmacological modulation of APP and TREM2 proteolysis has been and is tested in clinical trials and may lead to a causative treatment or prevention of AD [3,6]. Notably, although received with controversy in the field, immunotherapy-mediated removal of Aβ using the antibody aducanumab has recently been conditionally approved as the first causal therapy for AD in the U.S.

This review article focuses on the proteolytic processing of APP and TREM2. We will first introduce the proteases – referred to as secretases – that cleave APP and TREM2 and then

highlight recent discoveries on their genetic linkage to AD and their cellular regulation and provide an update on their suitability and use as drug targets for AD.

Secretases mediate APP processing and control Aβ generation

APP is a type I transmembrane protein and the precursor of the Aβ peptide (Fig. 2). Cleavage of APP by α-, β-, δ-, η-secretase results in secretion of the large extracellular APP domain (Fig. 2) [7]. This process is referred to as ectodomain shedding and occurs for hundreds of membrane proteins besides APP, including TREM2 [8]. In contrast, γ-secretase gradually cleaves APP within its transmembrane domain (TMD) thereby releasing 37-43 residue-long secreted Aβ peptides [9]. Depending on which proteases cleave APP, distinct proteolytic products are generated, which have different physiological functions or may contribute to AD [7] (Fig. 2).

In the following we focus on α-, β- and γ-secretase, which have been drug targets in clinical trials for AD and for which major new developments occurred during the past few years. The α-secretase activity cleaves APP in the middle of the Aβ domain, which precludes Aβ generation, and is predominantly mediated by 'a disintegrin and metalloprotease 10' (ADAM10) in the nervous system, but additional proteases such as the ADAM10-homolog ADAM17 may also contribute [10]. A pharmacological activation of APP cleavage by the α-secretase ADAM10 is feasible in vitro and in AD patients, as tested in a small phase 2a study [11,12], but safety and clinical benefit of this approach need to be tested in larger patient cohorts.

Processing of APP first by β- and then γ-secretase leads to Aβ generation (Fig. 2A). β-site APP cleaving enzyme 1 (BACE1) is the major β-secretase [13]. BACE2 is a homolog of BACE1, but cleaves within the Aβ domain of APP and can prevent Aβ generation [13,14]. Additional proteases, including meprin β and ADAMTS4, can cleave close to the BACE1 cleavage site and generate N-terminally truncated Aβ peptides (Fig. 2B), some of which such as Aβ4-x are abundant in amyloid plaques [15,16]. Interestingly, BACE1 can also cleave within the Aβ sequence after Aβ has been generated, which may constitute an Aβ clearance function that may be used to monitor AD progression [17]. γ-Secretase is a protein complex in the membrane consisting of four subunits, the proteolytically active subunit presenilin (PS) and the three non-proteolytic subunits nicastrin (NCT), anterior pharynx defective-1 (APH-1) and presenilin enhancer 2 (PEN-2) required for complex assembly, stabilization and maturation [9] (Fig. 2C). Numerous PS mutations were identified in dominantly inherited forms of AD,

which shift the major γ-secretase cleavage site from position 40 to 42 or in some cases also to 43 (Fig. 2D), thus resulting in longer Aβ peptides which aggregate more rapidly and form the major constituent of amyloid plaques [9].

Secretases cleave TREM2 and control its function in microglial phagocytosis

TREM2 undergoes cleavages by several secretases in microglia (Fig. 3). In a manner similar to APP, TREM2 can be cleaved in its ectodomain by ADAM10 and/or ADAM17 whereby the major extracellular part of TREM2 is removed [18-21]. The TREM2 ectodomain comprises the functionally important ligand-binding domain, which senses anionic lipids exposed by damaged or dying cells, myelin or Aβ [3]. An alternative ectodomain shedding cleavage by meprin β was reported to occur in macrophages [22]. Ectodomain shedding of TREM2 most likely is a major factor to cease cell-autonomous signaling, which is mediated by its membrane-bound binding partner DNAX-activation protein 12 (DAP12). Phosphorylation of DAP12 and subsequent binding of the kinase Syk induces downstream signaling pathways that control crucial microglial functions including microglial phagocytosis of loosely aggregated Aβ of amyloid plaque halos [3]. Removal of Aβ in the brain by this mechanism is stimulated by full-length (FL) TREM2 [3]. Certain mutations of TREM2 located in the ectodomain such as R47H, R62H or H157Y are associated with an increased risk of LOAD [3]. They functionally impact the ligand binding domain, interfere with maturation, ectodomain shedding or cell surface transport of TREM2 [18,20,21,23]. Similar to a knockout of TREM2, they have been shown to cause a loss of function (LOF) phenotype with impaired phagocytic activity [3].

The resulting TREM2 C-terminal fragments left in the membrane after ectodomain shedding can be further processed by γ-secretase thereby eventually terminating TREM2/DAP12-mediated signaling [18,24]. Cleavage occurs in a flexible region of its TMD after alanine A192 [25]. Interestingly, a mutation directly at the cleavage site (H157Y) has been identified in two Chinese AD patients (Fig. 3A) [26]. Its biochemical consequences have not yet been investigated but may yield interesting insights into the role of γ-secretase in TREM2 biology.

Genetics continues to provide novel insights into AD pathogenesis

Identification of mutated genes or genetic associations has been instrumental in unraveling the molecular and cellular causes of AD. For example, as for PS, numerous amino acid mutations were found in APP that cause dominantly inherited AD [27]. Most of these mutations occur outside of the Aβ sequence but in the vicinity of the β- and γ-secretase cleavage sites and generally enhance production of either the total amount of Aβ or specifically the long, more rapidly aggregating Aβ42 peptide, thus accelerating AD pathogenesis. Very recently, a new

mutation was identified next to the α-secretase cleavage site, which reduced cleavage of APP by α-secretase and increased its processing by β-secretase, resulting in enhanced Aβ generation. This Uppsala mutation results in deletion of amino acids 19-24 of the Aβ sequence of APP (Fig. 2E) [28]. Interestingly, the same region within Aβ was previously identified as a substrate inhibitory domain that suppresses Aβ generation [29]. In addition to its Aβ-increasing effect it also enhances Aβ aggregation, similar to single amino acid mutations identified previously within the Aβ sequence [27] (Fig. 2E) and provides further evidence for the central role of Aβ oligomers early in AD pathogenesis.

New genetic associations to AD were also reported for ADAM10 and ADAM17. One family with a nonsense mutation (Y167*) within the ADAM10 prodomain was identified. This predicted haploinsufficiency segregated with the disease and resulted in less α-secretase cleavage of APP, as determined by CSF analysis of APP cleavage products [30]. ADAM10 was also linked to AD through genome-wide association studies [31,32], but it remains unclear how the ADAM10 locus alters ADAM10 expression and whether it affects Aβ generation in neurons – similar to the nonsense mutation described above – or TREM2 shedding and phagocytosis in microglia, where ADAM10 is also expressed.

For ADAM17 a loss-of-function mutation (R215I) within the prodomain was associated with increased AD risk [33]. Mechanistically, this mutation was shown to enhance Aβ levels in vitro, but additional mechanisms cannot yet be ruled out and need to be explored. This is particularly relevant, because ADAM17 acts a) as a TREM2 sheddase and, thus, may alter microglial phagocytosis of Aβ, and b) cleaves TNF, thereby converting it to a pro-inflammatory, soluble cytokine, which is implicated in AD pathogenesis [34].

The role of TREM2 shedding in AD was further strengthened with the identification of the membrane-spanning 4-domains subfamily A (MS4A) gene cluster as a modulator of both soluble TREM2 and AD risk [35]. While that study revealed a colocalization of the MS4A protein MS4A4A with TREM2, further studies are needed to understand how this protein mechanistically controls TREM2 secretase cleavage.

Novel players regulating secretase activities

The past two years also provided important new insights into cellular mechanisms controlling secretase activity, in particular of the α-secretase ADAM10 and the β-secretase BACE1. ADAM10 activity is controlled at multiple levels [10,36]. Now, two additional ADAM10 inhibitory proteins - secreted frizzled-related protein 1' (Sfrp1) and the glycosylphosphatidylinositol

(GPI)-anchored membrane protein 'reversion-inducing cysteine-rich protein with Kazal motifs (RECK) - were directly linked to AD [37,38]. Both proteins were discovered to have enhanced abundance in AD brains, whereas genetic ablation of either protein enhanced ADAM10-cleavage of APP and reduced amyloid pathology in AD mice. Whether both proteins may be targeted pharmacologically in a safe manner remains to be studied.

For BACE1 exciting progress has been made in understanding its transcriptional regulation and the link thereof to AD. The gene expression regulatory protein ataxin-1 (Atxn1), which is linked to the neurodegenerative disorder spinocerebellar ataxia type 1, but also to AD, was found to repress BACE1 expression and control hippocampal neurogenesis and amyloid pathology in mouse brain [39]. Another transcription factor, 'nuclear factor erythroid-derived 2-related factor 2' (Nrf2), was also identified as a suppressor of BACE1 transcription [40]. Pathophysiological relevance of this Nrf2-dependent mechanism was established by demonstrating that NRF2 expression is reduced in AD brain and correlated with increased BACE1 protein levels.

BACE1 function may also be controlled by post-translational mechanisms, including by the intracellular trafficking protein γ-ear-containing ADP-ribosylation factor-binding protein 3 (GGA3). Last year, a loss-of-function mutation in GGA3 (Ins545T) was discovered that cosegregates with LOAD and mechanistically raises BACE1 abundance, leading to axonal trafficking disruption and axonal swellings in AD mice [41].

Therapeutic targeting of APP and TREM2 secretases

Proteases are classical targets for therapeutic interventions and this includes the Alzheimer secretases. Over the past few years, important new developments occurred for the BACE1 and γ-secretase-cleavage of APP and for the ADAM10/ADAM17-mediated shedding of TREM2.

Regarding BACE1 inhibition, six different BACE1-targeted small molecule inhibitors were tested until recently in phase 2 and 3 clinical trials. Although the drugs showed excellent target engagement and lowered Aβ in human brain and CSF, the trials were prematurely terminated because of futility, an unfavorable risk/benefit ratio or because of the occurrence of mild cognitive, but reversible worsening that is seen as an unacceptable side effect [42,43]. Analysis of the trial results has led to suggestions for future clinical trials, including the use of BACE inhibitors rather for prevention than for treatment of AD and a reduction of the inhibitor dose to avoid the side effects [6,13]. Another option to prevent the cognitive worsening may

be a cotreatment of BACE1 inhibitors together with activators of metabotropic glutamate receptor 1, which mitigated synaptic deficits upon BACE1 inhibition in mice [44]. An important task for the near future is to identify the molecular cause of the cognitive worsening, which is presumably caused by a too strong inhibition of cleavage of APP or another neuronal BACE1 substrate. Based on studies in adult mice, seizure protein 6, close homolog of L1, and an Aβ-independent proteolytic fragment of APP, referred to as Aηα, are likely candidates [45-47], but new BACE1 substrates continue to be identified [48-50], although their physiological BACE1-dependent functions have not yet been established. New BACE1 inhibitors are being developed and this does not only include small molecule drugs, but also BACE1-targeted antibodies [51].

γ-Secretase mediates the final cleavages in the generation of Aβ from APP and has thus been a major drug target since the discovery of PS as its catalytic component just over 20 years ago. γ-Secretase inhibitors (GSIs) have been abandoned due to severe side effects in clinical trials, which can at least in part be attributed to the inhibition of the generation of a key signaling molecule from Notch1, one of the most critical γ-secretase substrates. Instead of γ-secretase inhibition, modulation of the enzyme´s processivity activity in the generation of Aβ42/43 into presumably harmless shorter Aβ peptides such as Aβ38 by so-called γ-secretase modulators (GSMs) is considered a safe approach. Unlike GSIs, GSMs do not target the signaling cleavage functions of γ-secretase mediated by the intracellular domains of a number of other substrates besides Notch1. While the generation of these compounds has lacked behind the clinical development of BACE inhibitors, powerful GSMs with great promise for clinical trials are now emerging. One of these compounds combines high efficacy with an advanced safety profile in preclinical models [52]. Moreover, a novel GSM has been identified, which can also lower the pathogenic Aβ43 variant [53]. This and other GSMs are capable of breaking the resistance of a subset of PS FAD mutations in lowering Aβ42/43 informing the design of potential future GSM trials within FAD cohorts [54].

Another important step forward that may aid rational drug development are the recently obtained cryo-electron microscopy (cryo-EM) structures of γ-secretase in complex with APP or Notch1 substrates, different GSIs or a GSM at atomic resolution [55-57]. The GSM binding site was identified to locate on the extracellular side of PS in vicinity to its hydrophilic loop 1 and a nearby loop of the NCT ectodomain. This region had been implicated earlier as a site of GSM action [58,59]. While these structures might be a starting point for the development of improved APP-selective GSIs and could possibly lead to a revival of γ-secretase inhibition as a strategy for AD therapy, GSIs such as MRK-560 have been successfully repurposed in

preclinical models to interfere with enhanced Notch signaling in certain cancers such as T-ALL offering potential for further clinical development [60].

An additional drug target to modulate brain amyloidogenesis that has emerged in the past years is TREM2. Since its phagocytic activity towards Aβ is mediated by the non-shed FL protein at the cell surface, inhibition of TREM2 ectodomain shedding by ADAMs might be beneficial in AD. Because ADAM10 has numerous neuronal ADAM10 substrates [10,61,62], the possibility of specifically inhibiting TREM2 shedding by targeting its ADAM10/17 cleavage site was tested with an antibody binding near to the cleavage site at histidine 157 (Fig. 3) [19-21]. As a proof of concept, one antibody, 4D9, reduces TREM2 shedding, increases the levels of FL TREM2 and thereby increases intracellular signaling leading to microglial activation including enhanced removal of Aβ plaque halos in the brain of an AD mouse model [63]. Other antibodies raised against the TREM2 ectodomain that like 4D9 may all bind in the stalk region [3] showed similar microglial responses to AD pathology as 4D9 [64-66]. One of these antibodies, AL002c, that presumably like 4D9 blocks TREM2 shedding has already advanced into early phase clinical trials [65]. The relevance of TREM2 shedding for AD pathogenesis is further supported with the discovery of a LOF TREM2 mutation directly at the cleavage site (H157Y), which enhances shedding and reduces FL TREM2, causing reduced phagocytic activity and a genetic association to increased AD risk [20,21].

In addition to FL TREM2 signaling, which initiates a defense response to Aβ, the interaction of the shed soluble TREM2 ectodomain (sTREM2) with Aβ oligomers [67,68] may be protective against AD as well. As recently shown, sTREM2 cannot only bind Aβ oligomers but also disaggregate them and block Aβ-mediated neurotoxicity. The R47H AD risk variant is deficient in these activities and rather promotes Aβ aggregation into larger aggregates [69]. Notably, antibodies against the TREM2 ectodomain may also bind to sTREM2 [63,66]. Moreover, sTREM2 may have also signaling functions [70]. Thus, the therapeutic implications of reducing sTREM2 by antibody-mediated inhibition of FL TREM2 shedding require more research.

Conclusion

For many years, AD secretase research focused on the secretase substrate APP and its proteolytic conversion to Aβ. Although clinical trials with BACE1- and γ-secretase-targeted drugs did not (yet) lead to approval of AD drugs, interest in the AD secretases has strongly increased over the past few years, in particular with the discovery that the secretase-mediated shedding of TREM2 in microglia can be pharmacologically inhibited to enhance TREM2 function, for example in microglial phagocytosis of Aβ. First clinical trials targeting this

mechanism are ongoing or being prepared. Additionally, new, but safer clinical trials are now being considered with BACE1 inhibitors [6]. The detailed structural understanding of the mechanism of action of GSIs and GSMs may foster the development of new improved clinical candidates for AD trials. Another important area of research is the regulation of secretase activity, which may allow to identify new ways to therapeutically modulate secretase activity. Additionally, the detailed study of secretase substrates and functions beyond APP cleavage is intensively investigated, partly due to the desire to predict and understand mechanism-based side effects resulting from targeting secretase activity and partly driven by the basic research interest to understand the fundamental role of secretases in development, maintenance and function of the nervous system and other organs. Because secretases shed hundreds of single-span membrane proteins [8], it is likely that additional membrane proteins linked to AD will move to the center of secretase research in AD. This includes the LDL receptor-related protein 1 (LRP1), which is shed in the brain [48,71] and controls tau endocytosis and spreading between neurons [72], but potentially also the microglia-expressed AD risk genes CD33 and complement receptor 1 and the microglia-activation protein CLEC7a [2]. Taken together, we are in an exciting time for secretase research in AD which no longer focuses just on APP but also TREM2, and likely more AD-linked secretase substrates in the future.

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Declaration of interest

H.S. and S.K.T. declare no conflict of interest. S.F.L. has research collaborations on BACE inhibitors with Novartis and Shionogi.

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Selection of candidate highlight papers 2019, 2020, 2021

Papers of special interest (*):

- Bahn PNAS 2019: A signaling pathway involving the transcription factor Nrf2 is shown to suppress BACE1 transcription and ameliorate cognitive deficits in an Alzheimer's disease mouse model.
- Brummer EMBO Mol Med 2019: Using proteomics and CSF samples from a phase 2a clinical trial, this study reports that activation of the α-secretase ADAM10 may be feasible in a substrate-specific manner.
- Das Mol Psychiatry 2021: This study reports that positive allosteric modulators of metabotropic glutamate receptor 1 may alleviate cognitive worsening induced by clinically tested BACE1 inhibitors.
- Deming Sci Transl Med 2019: Using genetics and functional analysis, this study identified the MS4A gene cluster as a modifier of TREM2 shedding in Alzheimer's disease.
- Esteve Nat Neursoci 2019: This study demonstrates that the protein Sfrp1 increases Aβ generation in mice through inhibition of ADAM10 and that Sfrp1 protein abundance is enhanced in brains from Alzheimer patients.
- Nakamura Sci Transl Med 2019: Using mice, this study unravels a neuronal signaling pathway that controls ADAM10 activity and Aβ generation in mice.
- Pagnon de la Vega, Sci Transl Med 2021 in press: This study identifies the first deletion within the Aβ sequence of APP that causes dominantly inherited Alzheimer's disease.

Mechanistically, the mutation is shown to shift APP processing from α- to β-secretase and to modify Aβ aggregation.

- Petit EMBO J 2020: This paper shoes that a NCT loop region located in the vicinity of the hydrophilic loop 1 of PS contributes to the modulatory activity of GSMs on APP processing.
- Rynearson J Exp Med 2021: The authors report a novel GSM which shows promising safety and efficacy properties in preclinical models including non-human primates and thus has potential to advance to a safe clinical candidate for the treatment of AD.
- Suh Cell 2019: In mice loss of ataxin-1 enhances Alzheimer's disease pathogenesis through increasing transcription of the β-secretase BACE1.
- Trambauer EMBO Rep. 2020: This paper reports on the identification of a novel GSM which can also lower the pathogenic Aβ43 species that is particularly produced in higher amounts by a subset of PS FAD mutants.
- Vialta J Biol Chem 2021: This study describes an anti-aggregatory function of the shed sTREM2 for Aβ oligomers, which is compromised in TREM2 coding variants associated with increased AD risk.

Papers of outstanding interest (**):

- Price et al. J Neuroinflamm 2020: This paper shows that an agonistic antibody against the TREM2 ectodomain elicits microglial activation resulting in reduced amyloidosis and improved cognition in an AD mouse model.
- Schlepckow EMBO Mol Med 2020: This work shows that an agonistic antibody 4D9 against the TREM2 extracellular stalk region blocks ectodomain shedding and triggers microglial defense responses to amyloid pathology in an in vivo model of AD.
- Wang J Exp Med 2020: This study shows that the agonistic antibody AL002c against the ectodomain of human TREM2 induces microglial defense responses against amyloidosis that reduces the formation of neuritic plaque pathology in an AD mouse model. The authors further show that AL002c is safe and well-tolerated in a first phase I clinical trial.
- Fassler J Neuroinflamm 2021 The authors report microglial defense responses triggered by an antibody against the TREM2 ectodomain including improved cognition in an AD mouse model.
- Yang Cell 2021: By identifying the γ-secretase binding sites of GSIs of different functional types as well as that of a GSM at atomic resolution, the authors provide molecular bases for the mode of action of these compounds. The structures reveal that GSIs occupy the region of the APP and Notch1 substrate β-strands proving an

explanation of their inhibition mechanism, while the GSM binds at an extracellular PS/NCT interface.

- Zhou Science 2019: Groundbreaking structural analysis of a γ-secretase enzyme-substrate complex showing that the APP substrate forms a hybrid β-sheet with the enzyme whereby the initial substrate cleavage sites are unfolded. Basically identical substrate-binding features were published in parallel by the same group for Notch1 [54].

Figure Legends

Fig. 1: Amyloid cascade hypothesis of Alzheimer's disease. The amyloid cascade is a simplified model to illustrate key pathological changes during AD pathogenesis. Aβ is a proteolytic cleavage product of APP. Oligomeric Aβ aggregates induce neurotoxicity and chronic neuroinflammation and lead to aggregation of hyperphosphorylated forms of the microtubule-associated protein tau and finally to neurodegeneration and the onset of clinical AD symptoms. Aβ and tau aggregates grow over time, are used for the clinical diagnosis of AD upon autopsy and are then referred to as amyloid plaques and neurofibrillary tangles. Aβ has a short half-life and can be removed by microglia, which, in the course of AD pathology, switch from a homeostatic to an activated state. While Aβ phagocytosis by microglia reduces amyloid load, chronically activated microglia can also enhance the neuroinflammatory process.

Fig. 2: Proteolytic processing of APP and its role in AD. A-D. In the amyloidogenic pathway, APP processing by β- and γ-secretase generates Aβ (A) (marked in grey). β-Secretase activity is mediated by BACE1 and to a lower extent by meprin β, ADAMTS4 and potentially other proteases (B). γ-Secretase is a hetero-tetrameric protease complex consisting of the subunits shown in (C). γ-Secretase has different cleavage sites – predominantly after amino acid 40, but also 42 of Aβ - so that Aβ presents as a heterogenous peptide mixture including Aβ40 as most abundant peptide. Aβ42 is more hydrophobic, is the major constituent of amyloid plaques and is generated to a larger extent when γ-secretase is mutated in familial forms of AD (D). In the anti-amyloidogenic pathway, α-secretase cleavage – mediated by ADAM10 and related proteases – prevents Aβ generation. **E.** Familial AD mutations around the cleavage site of α-secretase may lower the anti-amyloidogenic APP processing and/or increase the aggregation propensity of Aβ. In contrast, the Aβ-lowering Icelandic mutation (blue) protects against AD. **F.** AEP/legumain acts as δ-secretase and may enhance β-secretase cleavage and Aβ generation. **G.** Processing of APP by η-secretase, followed by α- or β-secretase, generates the Aη peptide (Aηα, Aηβ), which is distinct from Aβ, but may exert neurotoxic functions in its Aηα variant form. η-Secretase activity may be mediated by MT5-MMP, but also by other, as yet unidentified proteases. For more details on APP processing, see text.

Fig. 3: Proteolytic processing of TREM2 and its role in Aβ phagocytosis. A. TREM2 processing pathways. TREM2 is cleaved in the stalk region of its ectodomain by ADAM10 and/or ADAM17. Alternatively, meprin β cleaves within the ectodomain more N-terminally. Both cleavages leave C-terminal fragments in the membrane which are subsequently cleaved by γ-secretase. The cleavages terminate TREM2 signaling, which is mediated by FL TREM2 in complex with DAP12 and leads to activation of homeostatic microglia thereby triggering protective activities including Aβ phagocytosis. The asterisk in the membrane-proximal stalk

region denotes the ADAM protease cleavage site at amino acid 157. A point mutation at this site, H157Y, which causes AD, enhances ADAM protease cleavage of TREM2, presumably reducing the protective TREM2 functions. **B.** Therapeutic targeting of TREM2 shedding. Agonistic antibodies such as 4D9 that prevent ectodomain shedding likely cause receptor cross-linking and trigger defense mechanisms to AD pathology from FL TREM2-mediated signaling.

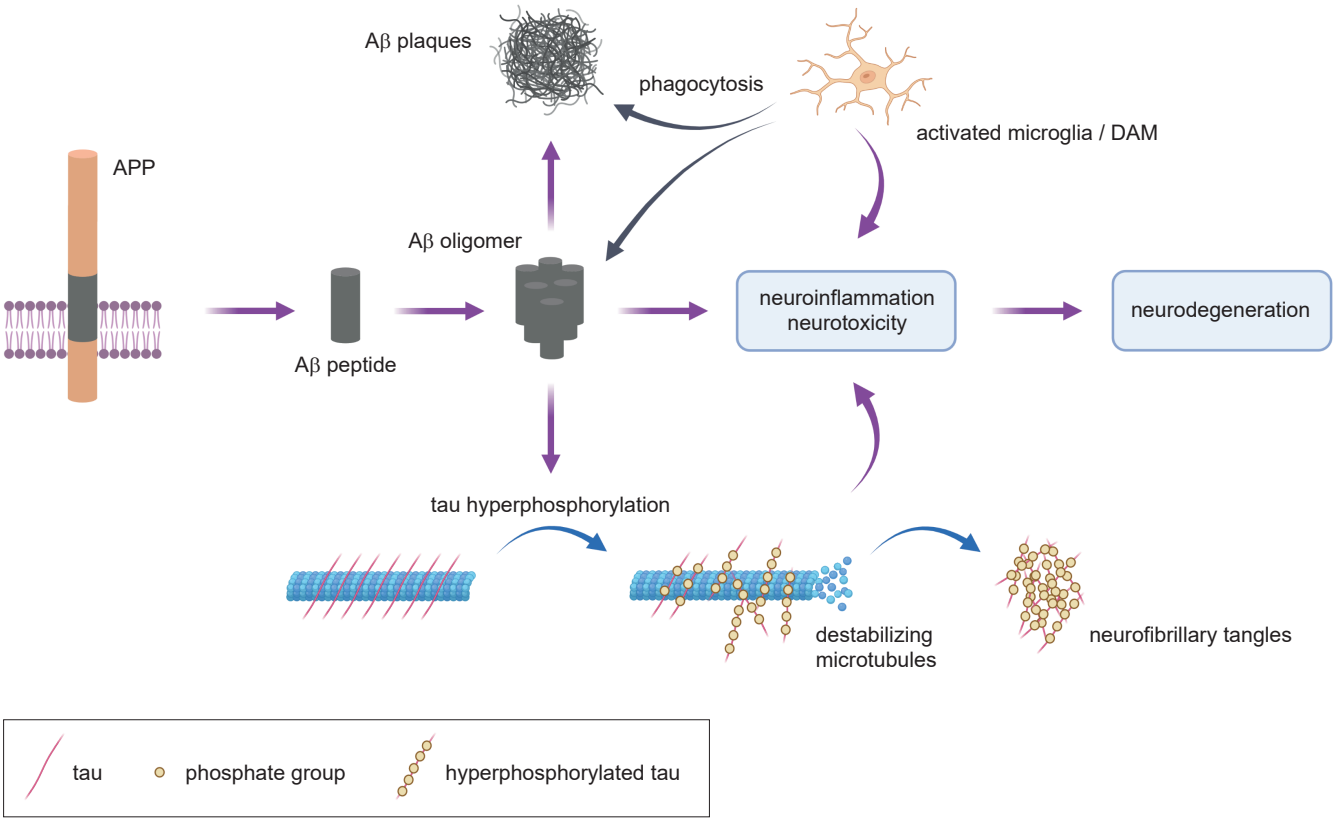
Aβ plaques
phagocytosis
activated microglia / DAM
APP
Aβ oligomer
Aβ peptide
neuroinflammation
neurotoxicity
neurodegeneration
tau hyperphosphorylation
destabilizing
microtubules
neurofibrillary tangles
tau
phosphate group
hyperphosphorylated tau

Fig. 1

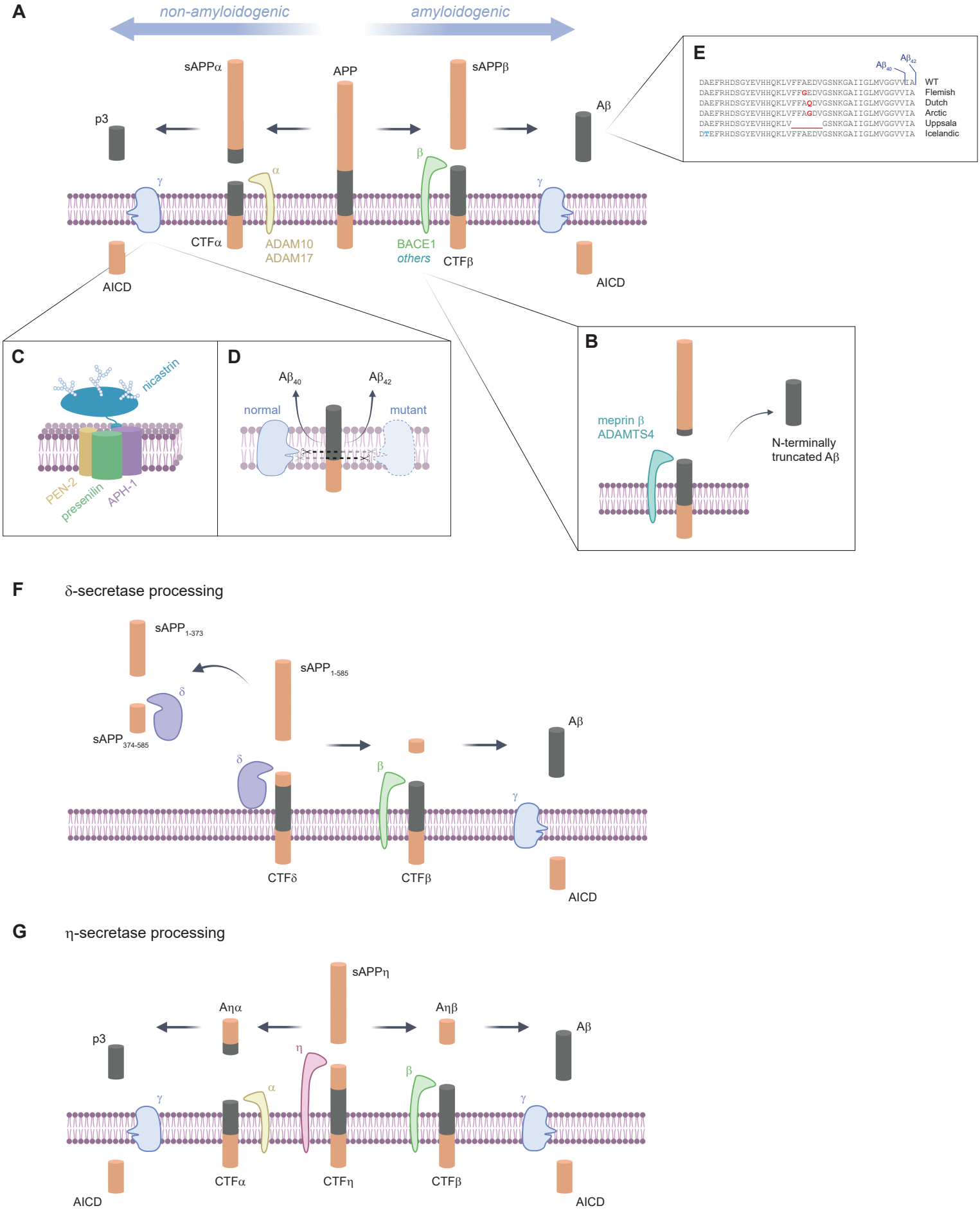
A
non-amyloidogenic
amyloidogenic
sAPPα
APP
sAPPβ
Aβ
p3
γ
α
β
γ
CTFα
ADAM10
ADAM17
BACE1
others
CTFβ
AICD
AICD
E
Aβ40
Aβ42
DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA WT
DAEFRHDSGYEVHHQKLVFFGEDVGSNKGAIIGLMVGGVVIA Flemish
DAEFRHDSGYEVHHQKLVFFAQDVGSNKGAIIGLMVGGVVIA Dutch
DAEFRHDSGYEVHHQKLVFFAGDVGSNKGAIIGLMVGGVVIA Arctic
DAEFRHDSGYEVHHQKLV_____GSNKGAIIGLMVGGVVIA Uppsala
DTEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA Icelandic
C
nicastrin
PEN-2
presenilin
APH-1
D
Aβ40
Aβ42
normal
mutant
B
meprin β
ADAMTS4
N-terminally
truncated Aβ
F
δ-secretase processing
sAPP1-373
sAPP1-585
δ
sAPP374-585
Aβ
δ
β
γ
CTFδ
CTFβ
AICD
G
η-secretase processing
sAPPη
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Fig. 2

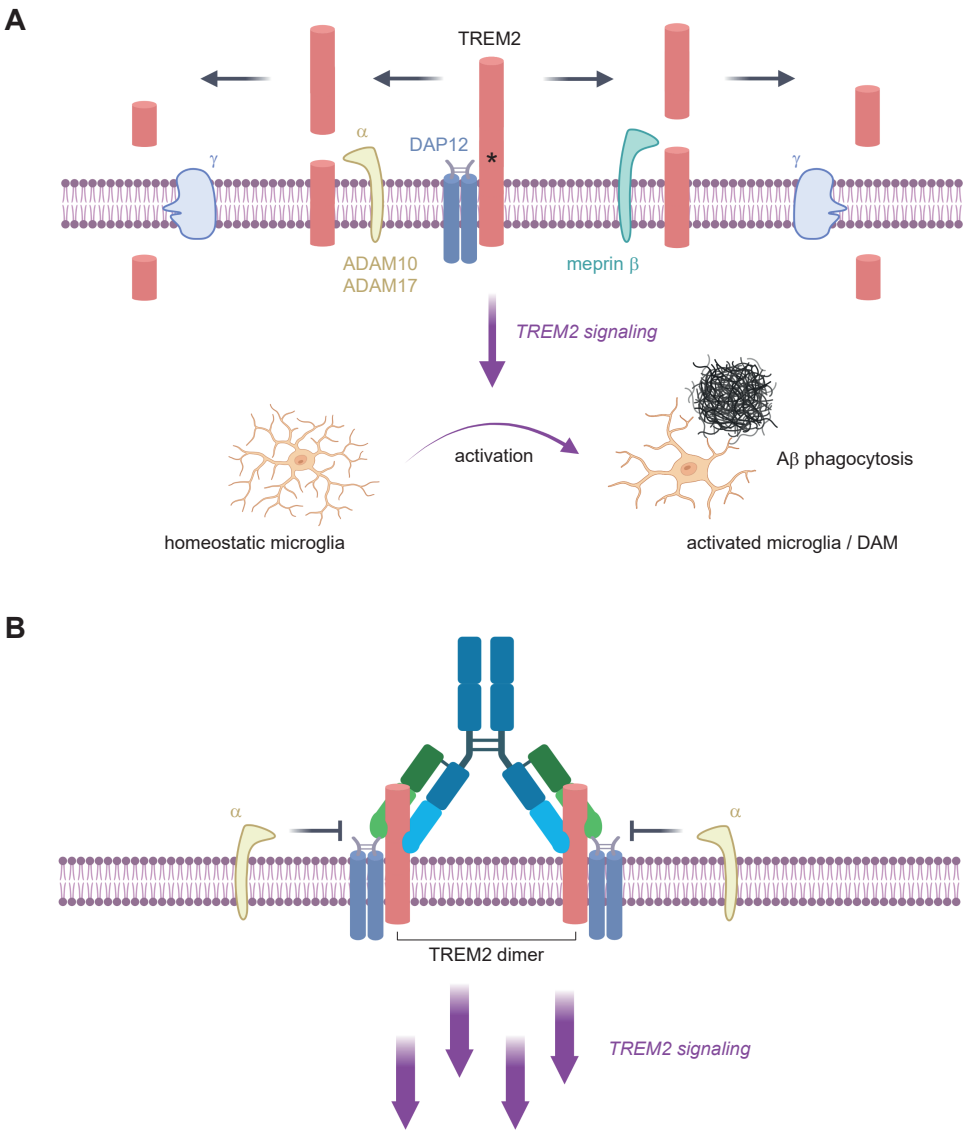
A
TREM2
α
DAP12
*
γ
γ
ADAM10
ADAM17
meprin β
TREM2 signaling
activation
Aβ phagocytosis
homeostatic microglia
activated microglia / DAM
B
α
α
TREM2 dimer
TREM2 signaling

Fig. 3