alzheimer's research&therapy

REVIEW

Molecular consequences of amyloid precursor protein and presenilin mutations causing autosomal-dominant Alzheimer's disease

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Abstract

Mutations in both the amyloid precursor protein (APP) and the presenilin (PSEN) genes cause familial Alzheimer's disease (FAD) with autosomal dominant inheritance and early onset of disease. The clinical course and neuropathology of FAD and sporadic Alzheimer's disease are highly similar, and patients with FAD constitute a unique population in which to conduct treatment and, in particular, prevention trials with novel pharmaceutical entities. It is critical, therefore, to exactly define the molecular consequences of APP and PSEN FAD mutations. Both APP and PSEN mutations drive amyloidosis in FAD patients through changes in the brain metabolism of amyloid-β (Aβ) peptides that promote the formation of pathogenic aggregates. APP mutations do not seem to impair the physiological functions of APP. In contrast, it has been proposed that PSEN mutations compromise γ-secretase-dependent and -independent functions of PSEN. However, PSEN mutations have mostly been studied in model systems that do not accurately reflect the genetic background in FAD patients. In this review, we discuss the reported cellular phenotypes of APP and PSEN mutations, the current understanding of their molecular mechanisms, the need to generate faithful models of PSEN mutations, and the potential bias of APP and PSEN mutations on therapeutic strategies that target Aβ.

Introduction

Alzheimer's disease (AD) is the most common age-related neurodegenerative disorder, currently affecting 20 to 30 million individuals worldwide [1]. The cardinal symptom of the disease is progressive memory loss due to the degeneration of neurons and synapses in the cerebral cortex and subcortical regions of the brain. Comprehensive evidence supports the amyloid hypothesis of AD, which argues that accumulation and aggregation of amyloid-β (Aβ) peptides in the brain is causal in its pathogenesis. Aβ is a proteolytic fragment generated through sequential cleavage of the amyloid precursor protein (APP) by β-secretase (BACE1) and γ-secretase. Cells produce Aβ peptides of variable length. A peptide of 40 amino acids (Aβ40) is the most prevalent species secreted by cells, whereas the longer Aβ42 isoform appears to be the key pathogenic species and the most abundant species deposited in the brain. Major support for the amyloid hypothesis is drawn from cases of early-onset familial AD (FAD). As used in this review, the term FAD is confined to familial cases with an autosomal-dominant inheritance pattern. Missense mutations that are causative of FAD have been identified in three genes that are essential for the generation of Aβ peptides: the *APP* gene and two homologous genes that encode the catalytic subunit of γ-secretase, *PSEN1* (encoding presenilin-1) and *PSEN2* (encoding presenilin-2) [2,3]. Overall, the clinical presentation of FAD patients with APP and PSEN mutations is very similar to that of sporadic AD, which is supported by neuroimaging, biomarker and post-mortem neuropathology studies. Recently, the clinical findings in FAD mutation carriers have been summarized in an excellent review by Bateman and colleagues [4] and will not be further discussed here.

Discovery of amyloid precursor protein mutations

It is undisputable that since the first description of the pathology of AD by the German psychiatrist and neuropathologist Alois Alzheimer in 1906 to the early days of the amyloid cascade hypothesis, both modern biochemistry and genetics have played major roles in advancing

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our understanding of this neurodegenerative condition. With the knowledge of the Aβ peptide sequence obtained from purified fractions of either vascular amyloid or senile plaques from AD and Down's syndrome patients, the identification of APP was a logical step forward [5-7]. Since the Aβ peptide represents only a small fragment of APP including part of the transmembrane domain, it was apparent that its *de novo* generation required at least two proteolytic activities (Figure 1a). Particularly troublesome at the time was the second processing step in the transmembrane domain (TMD) since intramembrane-cleaving proteases were only discovered more than a decade later. Nevertheless, with the sequencing of the Aβ peptide and the cloning of APP a lively scientific debate had begun on their causative association with AD. In addition to Aβ being the major constituent of two of the three hallmarks of AD - senile plaques and vascular amyloid - the chromosomal location of APP also strongly argued in favor of a crucial role for it. The *APP* gene is located on chromosome 21, which had been linked to AD by multiple genetic linkage studies and the observation that Down's syndrome patients develop dementia accompanied by prototypical neuropathological hallmarks of AD [8,9]. Three years after the cloning of APP, the E693Q Dutch missense mutation in the mid-region of Aβ (E22Q when referring to the Aβ peptide sequence) was identified as being causative of hereditary cerebral hemorrhage with amyloidosis Dutch-type (HCHWA-D) (Figure 1b) [10]. Although the neuropathology of HCHWA-D is clearly distinct from that of AD, this milestone discovery provided the first evidence that the *APP* gene harbors autosomal-dominant mutations causing dementia. HCHWA-D itself is characterized by severe vascular amyloid deposition, termed cerebrovascular amyloid angiopathy (CAA), in addition to parenchymal plaques. CAA as a result of targeting Aβ deposition to blood vessels ultimately leads to cerebral hemorrhages and strokes. One year later, the AD community received the long awaited news regarding the discovery of several causative FAD mutations in APP. These were located at the V717 position and included the London (V717I) [11], Indiana (V717F) [12] and V717G [13] mutations. These major discoveries spurred the identification of a continuous stream of additional CAA and FAD mutations to the present time (highlighted in Figure 1b and Table 1). The detailed investigation of their biological phenotypes relied, to a large extent, on progress in the understanding of the physiological metabolism of APP and further advancements in assay technologies, such as highly sensitive immunoassays capable of discriminating Aβ40 and Aβ42 peptides. In this respect, the observation that Aβ peptide secretion is the result of a physiological process not only pointed towards the existence of cellular proteases capable of APP processing but also provided an *in vitro* system for studying mutant APP variants in detail [14].

Table 1. Primary references of amyloid precursor protein mutations

Mutation[a]	Phenotype	Common name	Publication date	Reference
KM670/671NL	AD	Swedish	1992	[38]
A673V	AD (recessive)		2009	[105]
H677R	AD		2003	[106]
D678N	AD		2004	[107]
A692G	CAA[b]	Flemish	1992	[108]
E693Q	HCHWA-D[b]	Dutch	1990	[10]
E693G	AD	Arctic	2001	[26]
E693K	CAA	Italian	2010	[109]
E693Δ	AD	Osaka	2008	[27]
D694N	AD and CAA	Iowa	2001	[110]
A713T	AD and CAA		2004	[111]
T714I	AD	Austrian	2000	[112]
T714A	AD	Iranian	2002	[113]
V715M	AD	French	1999	[114]
V715A	AD	German	2001	[115]
I716V	AD	Florida	1997	[116]
I716F	AD		2010	[117]
V717I	AD	London	1991	[11]
V717F	AD	Indiana	1991	[12]
V717G	AD		1991	[13]
V717L	AD		2000	[118]
T719P	AD		2009	[119]
L723P	AD	Australian	2000	[120]
K724N	Likely AD		2006	[121]

[a]Numbering according to the position in the APP 770 isoform. [b]CAA, cerebral amyloid angiophathy; HCHWA-D, hereditary cerebral hemorrhage with amyloidosis Dutch-type (for a continuously updated list of APP mutations, see [55]). AD, Alzheimer's disease; APP, amyloid precursor protein.

Amyloid precursor mutations causative of cerebrovascular amyloid angiopathy

When comparing the APP FAD and CAA mutations it is evident that these cluster around hot spots in the APP protein sequence (Figure 1a). The mutations causative of CAA are located in the central region of the peptide. At the molecular level, these mutations change the charge distribution and thereby likely affect the peptide structure, ultimately promoting fibril formation [15,16]. Most data to date have been generated for the E22Q (E693Q) Dutch peptide. Limited proteolysis and NMR has identified a turn in the V24-K28 region, which appears to be critical for folding of the monomer and is stabilized, in part, by electrostatic interactions between

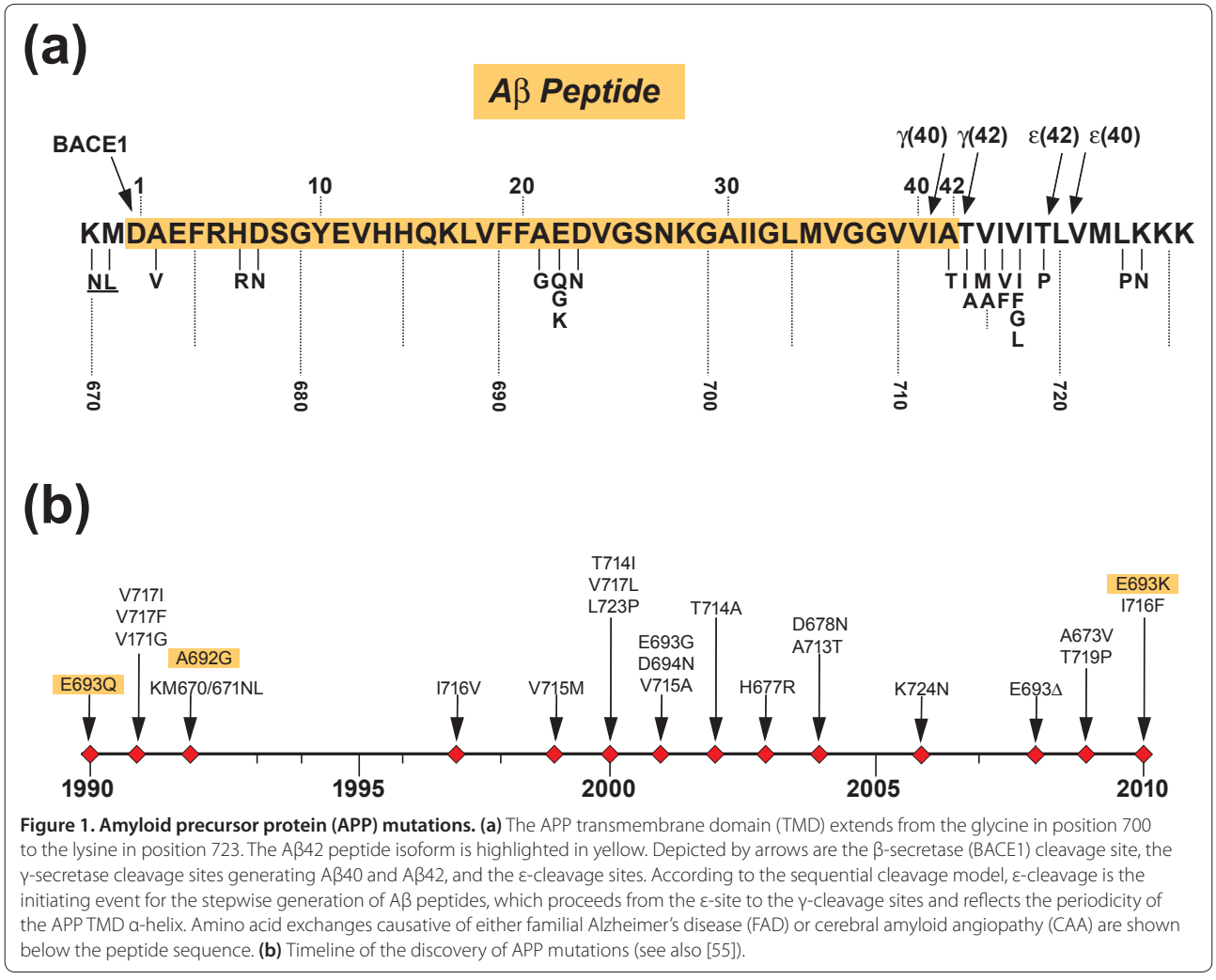

Figure 1. Amyloid precursor protein (APP) mutations. (a) The APP transmembrane domain (TMD) extends from the glycine in position 700 to the lysine in position 723. The Aβ42 peptide isoform is highlighted in yellow. Depicted by arrows are the β-secretase (BACE1) cleavage site, the γ-secretase cleavage sites generating Aβ40 and Aβ42, and the ε-cleavage sites. According to the sequential cleavage model, ε-cleavage is the initiating event for the stepwise generation of Aβ peptides, which proceeds from the ε-site to the γ-cleavage sites and reflects the periodicity of the APP TMD α-helix. Amino acid exchanges causative of either familial Alzheimer's disease (FAD) or cerebral amyloid angiopathy (CAA) are shown below the peptide sequence. **(b)** Timeline of the discovery of APP mutations (see also [55]).

E22 and K28. E22 and D23 mutations destabilize this turn and thereby promote oligomer formation [17,18]. Accordingly, in biological systems, increased toxicity in human leptomeningeal smooth muscle cells and enhanced neurotoxicity have been reported for the Dutch peptide [19,20]. This peptide also appears to be less efficiently degraded by the prototypical Aβ-degrading enzyme insulin-degrading enzyme [21]. Transgenic animal models expressing Dutch APP recapitulate the human pathology, with the vasculature being the main site of amyloid deposition [22]. With respect to Aβ production itself, no coherent phenotype has been observed for the CAA mutants. The A692G Flemish mutation enhances Aβ40 and Aβ42 production whereas the Dutch mutation does not appear to affect Aβ production at all [23]. The enhanced Aβ production of the Flemish mutation was reported in 1994 [24]. Many years later, a systematic analysis of the domain surrounding A692 has identified a substrate inhibitory domain (ASID) in APP [25]. This domain appears to exert a negative control over the activity of γ-secretase. Intriguingly, only the A692G amino acid exchange introduced by the Flemish mutation, but none of the other CAA/FAD-associated mutations in the ASID domain, lowered its inhibitory potency, thus raising Aβ production [25]. This adds another facet to the mechanistic understanding of the Flemish CAA mutation. It is reasonable to assume, however, that the main driver for the CAA pathology is the change of the Aβ peptide sequence itself, since increasing total Aβ production will lead to FAD and not CAA, as shown for the APP Swedish mutation (see below).

It is important to note that not all mutations in the central region of Aβ cause CAA, as highlighted by the E693G Arctic and the E693Δ FAD mutations [26,27]. Despite changing the sequence at exactly the same position affected by the Dutch and Italian CAA mutations, the Arctic mutation causes FAD characterized by the abundance of parenchymal plaques. However, these deposits are mainly ring-like in shape and devoid of a

dense core [28]. In good agreement with the human pathology, transgenic mouse models overexpressing Artic APP show fast and extensive parenchymal plaque formation and lack the profound vascular pathology observed in Dutch APP-overexpressing mice [29]. Conflicting data have been published for the APP E693Δ FAD mutation. This mutation was initially reported to promote the formation of toxic oligomers, and APP E693Δ transgenic mice lack extensive amyloid deposition [27,30]. In contrast, more recent biophysical studies have shown that the mutant peptide forms amyloid fibrils extremely rapidly [31-33].

Taken together, this leaves some important questions unanswered, such as why CAA mutations specifically target Aβ deposition to the brain capillaries. One common hypothesis is that the aggregation kinetics of the CAA peptides reduce their clearance across the blood-brain barrier [22]. A major contributor could be the specific cellular environment in the vasculature since smooth muscle cell surfaces in particular have been shown to promote CAA Aβ aggregation [34].

Amyloid precursor protein mutations causative of familial Alzheimer's disease

In retrospect it is not surprising that almost all APP mutations causative of FAD cluster around the sites of proteolytic processing by the β-secretase and γ-secretase enzymes, releasing the Aβ peptides into the luminal/extracellular compartment. A seminal observation came from the analysis of the KM670/671NL Swedish APP mutation, which reproducibly increased total Aβ secretion in both Swedish APP transfected cells and skin fibroblasts from carriers of the mutation [35-38]. Mechanistically, this mutant is well understood, converting the APP sequence into a better substrate for BACE1, which became apparent after the enzyme had been cloned [39,40]. This increase in substrate affinity not only raises Aβ production but also influences the cellular compartment where the cleavage takes place. Whereas BACE1 processing of wild-type (WT) APP requires trafficking to the cell surface and recycling into early endosomes [41], evidence from non-neuronal cell lines suggests that Swedish APP may already be processed in the trans-Golgi network compartment [42]. Both of these unique features of Swedish APP have therapeutic implications. Since all BACE1 inhibitors currently entering clinical development target the active site, these can be presumed to be competitive with the substrate. This has consequences for their pharmacology and compound affinities are reduced in Swedish APP-expressing systems [43]. Consequently, BACE1 inhibitor drugs could be less efficient at inhibiting BACE1 in Swedish APP mutation carriers. In addition, if antibodies inhibiting BACE1 were to be moved into the clinic, it is unlikely that these would reach the early intracellular compartments where Swedish APP is cleaved. This was supported by a recent study demonstrating that, in contrast to the situation in WT animals, BACE1 antibodies were incapable of inhibiting the enzyme in a Swedish APP transgenic mouse model [44].

The remaining FAD mutations tend to accumulate distal to the γ-secretase cleavage site. Mechanistically, most of them elevate the Aβ42/Aβ40 ratio (Table 2), with the most robust data being obtained for the V717 FAD mutants [45-47]. This strongly supported a causative role of the longer Aβ42 peptide, which in animal models appeared to be essential for senile plaque formation [48]. However, the discovery of the ε-cleavage [49], which leads to the release of the APP intracellular domain (AICD), suggested that aberrant APP/AICD signaling might provide an alternative explanation of how APP FAD mutations cause AD. The ε-cleavage is homologous to the S3 cleavage in the Notch receptor and occurs in proximity to the cytosolic face of the membrane. It is also mediated by γ-secretase and liberates an intracellular domain capable of recruiting accessory proteins, which in turn could modulate nuclear gene expression [2]. When AICD generation from FAD mutants was quantified, conflicting data were obtained depending on the assay used (Table 2). Using a luciferase reporter assay in cells essentially reflecting AICD detachment from the membrane and translocation to the nucleus, several APP FAD mutants did not show any differences compared to the WT APP [45]. When AICD generation in membranes was quantified by western blot immunodetection, some mutations (for example, T714I) showed reduced and some increased (for example, I716V) AICD production, whereas all FAD mutants increased the Aβ42/Aβ40 ratio [46]. Overall, despite some experimental differences, no coherent pattern has been reported for AICD generation and APP mutations. It is not likely, therefore, that disturbed APP/AICD signaling contributes to FAD.

The key question of how exactly these FAD mutations promote the elevation of the Aβ42/Aβ40 ratio still remains unresolved. The answer may lie in the way γ-secretase cleaves its substrates. γ-Secretase cleaves at multiple sites within the APP TMD, and various Aβ peptide species have been identified in cell supernatants (Aβ33, 34, 37, 38, 39, 40, 42, 43) and cell lysates (Aβ45, 46, 48, 49). Recent data suggest a stepwise mode of cleavage with initiation at the ε-cleavage site [2,50]. This initial processing event is followed by successive tripeptide generation, which proceeds from the ε-cleavage site to the γ-cleavage sites and reflects the periodicity of the α-helix. According to this model, the initiation site for Aβ42 and Aβ40 would be at positions 48 (APP T719) and 49 (APP L720), respectively, in the Aβ domain. An increase in the efficiency to initiate the Aβ42 lineage of

Table 2. Phenotypes of common amyloid precursor protein mutations

APP mutation	Aβ generation	AICD generation	AICD/ε-cleavage quantification	Reference
KM670/671NL (Swedish)	Total Aβ ↑ in transfected cells (6- to 8-fold) and human FAD fibroblasts (3-fold)	ND	ND	[35-37]
Various 717	Aβ42 ↑ (1.5- to 1.9-fold)	ND	ND	[47]
T714I, V715M, I716F, V717I, V717F, V717G	Aβ42/40 ↑ for V717I, others ND	AICD →	C99 luciferase reporter	[45]
T714I, V715M, I716V, V717I, V717L, L723P	All mutants Aβ42/Aβ40 ↑	T714I: AICD ↓ L723P: AICD ↓ V715M: AICD ↑ I716V: AICD ↑ V717I : AICD → V717L: AICD →	Immunodetection of AICD after *in vitro* generation in membranes (APP Swedish combined with second mutant)	[46]
T714I	Aβ42/Aβ40 ↑ (11-fold)	ND	ND	[113]
V715F	Aβ40 and Aβ42 ↓ Aβ38 ↑	AICD →	Immunodetection of AICD after *in vitro* generation in membranes	[123]
E693Q Dutch	Aβ →	ND	ND	[23]
A692G Flemish	Aβ40 and Aβ42 ↑	ND	ND	[23,24]
E693G Arctic	Aβ42 ↓	ND	ND	[26]
A673V (recessive)	Aβ40 and Aβ42 ↑ Aggregation and fibril formation ↑ in homozygous carriers, but anti-amyloidogenic in heterozygous	ND	ND	[105]

Note that immunoassays discriminating Aβ40 and Aβ42 became available in 1994 [46] and any prior data are based on immunoprecipitation of ^{35}S-methionine labeled total Aβ. ε-Cleavage leading to AICD formation was discovered in 2001 [48]. Up arrows indicate increase; down arrows indicate decrease; right-pointing arrows indicate no change compared to WT APP. Aβ, amyloid-β; AICD, amyloid precursor protein intracellular domain; APP, amyloid precursor protein; ND not determined.

peptides at T719 or in turn a decrease at initiating the Aβ40 lineage at L720 would lead to an elevation of the Aβ42/Aβ40 ratio. In this respect, the region comprising residues T714 to V717 must harbor critical structural determinants governing enzyme binding and positioning for lineage initiation [51]. Mechanistically, one could view these mutations as quasi loss-of-function variants. If the enzyme had evolved to efficiently convert the APP substrate into Aβ40, any mutation forcing the enzyme towards the less efficient Aβ42 lineage would fit this definition.

Presenilin mutations

The vast majority of FAD cases harbor heterozygous mutations in the *PSEN1* gene on chromosome 14. Sherrington and colleagues [52] identified the first mutations in *PSEN1* in 1995. In the same year, mutations in the homologous gene *PSEN2*, on chromosome 1, were described [53,54]. Since then, more than 180 different pathogenic mutations in more than 400 families have been identified in *PSEN1* and an additional 13 mutations in *PSEN2* (see the Alzheimer Disease and Frontotemporal Dementia Mutation Database [55,56] for a complete list of mutations). Individuals with *PSEN1* mutations typically become symptomatic between the ages of 30 and 50 years.

γ-Secretase-dependent and -independent functions of presenilin proteins

PSEN proteins have been proposed to exert both γ-secretase-dependent and -independent functions. While it is far beyond the scope of this review to discuss all known physiological functions of PSEN proteins, we will briefly summarize PSEN activities that might be impaired by FAD mutations.

PSEN proteins form the catalytic core of γ-secretase, a multi-subunit aspartyl protease that catalyzes the last step in the generation of the Aβ peptides from its substrate APP [2]. PSEN proteins have a nine TMD topology, and two critical aspartate residues in TMD6 and TMD7 form the active center of γ-secretase. PSEN proteins are incorporated together with three accessory proteins, nicastrin, APH-1 (anterior pharynx defective-1), and PEN-2 (presenilin enhancer-2), into high molecular weight complexes. In addition to APP, more than 90 type-I transmembrane proteins have been identified as substrates of γ-secretase. The most prominent substrate aside from APP is the NOTCH receptor. Processing of

NOTCH by γ-secretase liberates the NOTCH intracellular domain (NICD), which translocates into the nucleus and regulates transcription of target genes involved in cell fate decisions during embryogenesis and adulthood. Abrogation of NOTCH receptor processing and signaling causes dramatic phenotypes in a variety of organisms [2]. Another example is the proteolytic processing of the transmembrane receptors ErbB4 and DCC (deleted in colorectal cancer) by γ-secretase, which appears to be required for important neurodevelopmental processes such as axon guidance and astrogenesis [57,58]. However, the physiological significance of γ-secretase-mediated cleavage events in most of the other substrates remains to be clarified.

PSEN proteins may also have important γ-secretase-independent functions. These include the modulation of specific signal transduction pathways, a critical function in lysosomal proteolysis and autophagy, and the regulation of the cellular calcium homeostasis [2,59-62].

Current models of FAD-associated presenilin mutations

It is generally accepted that the effects of APP mutations on Aβ generation and aggregation can be accurately modeled by overexpression of mutant APP in cultured cells or transgenic mice; however, this is less evident for PSEN mutations. Overexpression of PSEN does not increase γ-secretase activity or production of Aβ peptides by itself. Instead, ectopic expression of PSEN leads to incorporation of exogenous PSEN molecules into the complex in exchange for endogenously expressed PSEN [63]. This replacement phenomenon demonstrates that the active γ-secretase complex contains all four subunits in a definite stoichiometry, and that the abundance of the accessory proteins is limiting for the formation of mature and enzymatically active complexes [2]. In most studies, FAD PSEN mutants have been stably overexpressed in permanent cell lines or transgenic mice, leading to replacement of endogenous WT PSEN1 and PSEN2 proteins in cellular γ-secretase complexes [63]. Alternatively, PSEN mutants were expressed in *PSEN1*/*PSEN2*-/- double-knockout cell lines that do not harbor endogenous WT PSEN proteins [64,65]. Accordingly, it is expected that functional γ-secretase complexes in both of these tissue culture models contain predominantly or solely the exogenously expressed PSEN mutants (Figure 2). However, this situation is strikingly different from FAD patients with heterozygous *PSEN1* (or *PSEN2*) mutations who express mutant and WT PSEN1 (or PSEN2) in an approximately equal ratio in the background of two WT *PSEN2* (or *PSEN1*) alleles (Figure 2). In addition, a small number of knock-in mouse strains for FAD *PSEN1* mutations have been created, in which the mutant alleles are expressed under control of the endogenous mouse *PSEN1* promoter, and few studies have investigated the impact of the mutant alleles on Aβ production, processing of other γ-secretase substrates, and γ-secretase-independent functions of PSEN [66-69]. Finally, some studies have used primary cells from FAD patients to confirm proposed effects of PSEN mutants [70]. Nevertheless, it follows that the vast majority of investigations have been conducted in model systems that seem appropriate to assess the effects of isolated mutant alleles but that do not accurately reflect the genetic background in FAD patients with PSEN mutations.

Presenilin mutations: loss-of-function, gain-of-function, or both?

A vigorous debate has been initiated over the issue of whether FAD PSEN alleles represent loss-of-function or gain-of-function mutations. The arguments in this debate range from the proposition that alterations in the Aβ42/Aβ40 ratio are the only meaningful outcome of PSEN mutations to the hypothesis that AD is unrelated to changes in Aβ production and is primarily caused by a loss of various PSEN protein functions [71-73]. γ-Secretase-dependent phenotypes of specific PSEN mutations that have been investigated in multiple independent studies or model systems are summarized in Table 3.

Initially, measurements of steady-state Aβ levels in transfected cells, transgenic mice and primary cells of FAD patients with PSEN1 or PSEN2 mutations suggested that the common pathogenic mechanism of PSEN mutations was to selectively elevate the absolute amount of cellular Aβ42 production, which was interpreted as a gain-of-toxic function mechanism [70,74]. However, subsequent experiments have demonstrated that many FAD PSEN mutations when overexpressed display reduced overall γ-secretase activity compared to WT PSEN proteins. This was first recognized by Song and colleagues, who showed that overexpression of the PSEN1 mutations C410Y and G384A in *PSEN1*-/- knockout cells resulted in reduced NICD production [75]. These findings correlated closely with results from *in vivo* experiments in PSEN-deficient *Caenorhabditis elegans* and *Drosophila* that reported a complete rescue of NOTCH phenotypes after transgenic expression of human WT PSEN1 but only partial rescue with FAD PSEN1 mutants [76,77].

Since then several studies have confirmed that many PSEN1/PSEN2 mutations cause reduced NOTCH cleavage, decreased formation of the AICD fragment, and reduced processing of other γ-secretase substrates such as N-cadherin [64,65,78-81]. Studies have further shown that elevations in the Aβ42/Aβ40 ratio after expression of some PSEN mutations are, to a large degree, due to reduced Aβ40 levels and not to increases in the absolute amounts of Aβ42 peptides [64,65,69,80,82-84]. In the majority of these studies, PSEN mutations

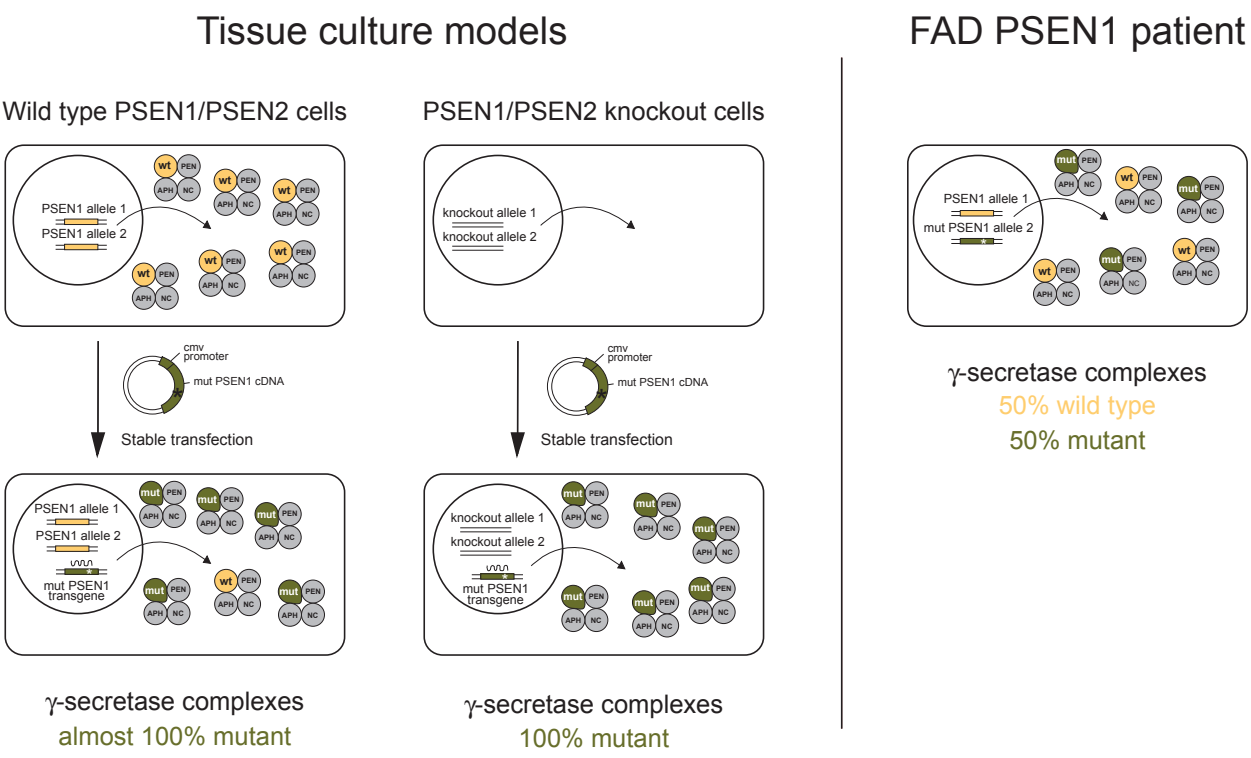

Figure 2. Tissue culture models of presenilin (PSEN) mutations. In most studies, PSEN mutants have been stably overexpressed either in permanent cells lines (left) or in *PSEN1*/*PSEN2*-/- double-knockout cell lines (middle). Due to the replacement phenomenon or the lack of endogenous wild type (WT) PSEN proteins, functional γ-secretase complexes in both of these tissue culture models contain predominantly or solely the exogenously expressed PSEN mutants. This situation is different from familial Alzheimer's disease (FAD) patients with heterozygous *PSEN1* (or *PSEN2*) mutations that express mutant and WT PSEN1 (or PSEN2) in an equal ratio in the background of two WT *PSEN2* (or *PSEN1*) alleles (right). CMV, cytomegalovirus.

were stably or transiently overexpressed in either permanent cell lines with endogenous PSEN expression or in fibroblasts derived from *PSEN1*/*PSEN2*-/- knockout mice, and steady-state levels of Aβ peptides and γ-secretase cleavage product were measured in cell culture supernatants or lysates. With regard to Aβ, these steady-state measurements represent the net effect of production, degradation, secretion and cellular uptake. In addition, kinetic studies of PSEN mutants have been performed in cell-free assays, which use solubilized membrane preparations from cells expressing PSEN mutants and employ exogenously added recombinant APP carboxy-terminal fragments (CTFs) as substrates. These assays have confirmed that the rate of production of Aβ peptides and the AICD domain over time is reduced for some PSEN mutants compared to WT PSEN [69,82-84]. The one consistent feature of PSEN mutations in all of these studies is that they increase the Aβ42/Aβ40 ratio. For the well-studied PSEN mutations listed in Table 2, this change in the Aβ42/Aβ40 ratio can be due to an increase in Aβ42 levels with unchanged Aβ40 (PSEN1-M146L), increased Aβ42 with decreased Aβ40 (PSEN2-N141I), unchanged Aβ42 with decreased Aβ40 (PSEN1-I213T), or a decrease in both Aβ42 and Aβ40 (PSEN1-C410Y). In addition, the mutants impair AICD and NICD generation and processing of other γ-secretase substrates like N-cadherin to variable degrees. PSEN mutants that lower Aβ40 levels, such as PSEN1-L166P and PSEN2-N141I, tend to impair AICD and NICD generation, indicating a more severe loss of γ-secretase enzyme activity. In contrast, mutants that preserve Aβ40 levels, such as PSEN1-M146L and PSEN1-A246E, also appear to preserve AICD and NICD levels, which is reflected in the ability of the PSEN1-A246E mutation to fully rescue the lethal phenotype of *PSEN1*-/- knockout mice [85,86]. Overall, results from cell-based models with overexpression of PSEN mutants and of kinetic studies in cell-free assays have been reasonably consistent in demonstrating a gradual loss of γ-secretase activity with the effect size depending on the specific PSEN mutation (Table 3). How can an overall decrease in γ-secretase activity caused by PSEN mutations explain the observed increase in the Aβ42/Aβ40 ratio? According to the sequential cleavage model of Aβ generation, γ-secretase cleavage takes place

Table 3. γ-Secretase-dependent phenotypes of presenilin mutations

Model system	Aβ40	Aβ42	Aβ42/Aβ40	AICD	NICD	N-cadherin	Other phenotypes	Reference
PSEN1-M146L								
Overexpression (HEK293, CHO)	→	↑	↑	ND	ND	↓	-	[79,80,83]
Overexpression (PSEN-/-)	ND	ND	ND	ND	→	ND	-	[81]
Kinetic *in vitro* assay	→	↑	↑	ND	ND	ND	-	[83]
Transgenic mice	ND	↑	↑	ND	ND	ND	Total Aβ →	[74]
PSEN1-L166P								
Overexpression (HEK293)	↓	↑	↑	↓	↓	ND	-	[80]
Overexpression (PSEN-/-)	↓	→	↑	↓	↓	↓	APP-CTFs ↑	[64]
PSEN1-I213T								
Overexpression (PSEN-/-)	↓	↓	↑	ND	ND	ND	APP-CTFs →	[123]
Kinetic *in vitro* assay	↓	→	↑	ND	ND	ND	-	[84]
Knock-in mice (heterozygous)	→	↑	↑	ND	ND	ND	-	[67]
PSEN1-R278I								
Knock-in mice (heterozygous)	↓[a]	→	→	→	→	→	APP-CTFs →	[68]
Primary cells (from knock-in mice)	↓	→	↑	ND	→	ND	Total Aβ → Aβ43 ↑	[68]
Kinetic *in vitro* assay	↓	↓	ND	↓	ND	ND	Total Aβ ↓	[68]
PSEN1-A246E								
Overexpression (PSEN-/-)	→	→	↑	↓	→	→	APP-CTFs →	[64,81]
Transgenic mice	→	↑	↑	ND	ND	ND	-	[124]
Primary cells	↑	↑	↑	ND	ND	ND	-	[70,125]
PSEN1-ΔExon9								
Overexpression (HEK293)	→	↑	↑	↓	↓	↓	-	[78-80]
Overexpression (PSEN-/-)	↓	→	↑	↓	↓	↓	APP-CTFs ↑	[64,81]
Transgenic mice	→	↑	↑	ND	ND	ND	-	[124]
PSEN1-G384A								
Overexpression (HEK293, CHO)	↓→	↑	↑	ND	ND	↓	-	[79,80,83]
Overexpression (PSEN-/-)	↓→	↑	↑	↓	↓	↓	APP-CTFs →	[64,75,107]
Kinetic *in vitro* assay	↓	→↑	↑	↓	ND	ND	Total Aβ ↓	[82,83]
PSEN1-C410Y								
Overexpression (PSEN-/-)	↓	↓	↑	ND	↓	ND	APP-CTFs ↑	[75,81,123]
PSEN2-N141I								
Overexpression (COS-1, N2a, CHO)	↓	↑	↑	ND	ND	ND	-	[83,126]
Overexpression (PSEN-/-)	↓	↑	↑	↓	↓	↓	APP-CTFs →	[64,65]
Kinetic *in vitro* assay	↓	↑	↑	ND	ND	ND	-	[83]
Transgenic mice	↓	↑	↑	ND	ND	ND	-	[127,128]
Primary cells	→↑	↑	↑	ND	ND	ND	-	[70,125]

PSEN mutations were chosen based on their investigation in multiple independent studies and model systems. All phenotypes of the PSEN mutants are reported in comparison to WT PSEN1. Studies that did not include a WT PSEN control condition are not included in this table. Up arrows indicate increase; down arrows indicate decrease; right-pointing arrows indicate no change compared to WT PSEN. Two arrows next to each other indicates that two or more studies reported different results compared to WT PSEN. Increased APP-CTFs, which are the immediate substrates of γ-secretase, can be interpreted as a sign of reduced enzyme activity. N-cadherin processing by γ-secretase was assessed in the studies by Bentahir and colleagues [63], Marambaud and colleagues [78] and Saito and colleagues [67] in different ways. However, in all cases a decrease indicates reduced processing of N-cadherin and diminished formation of the N-cadherin intracellular domain. [a]The reduction in endogenous mouse Aβ40 steady-state levels in brain of heterozygous knock-in mice for the PSEN1-R278I mutation was only observed in the guanidine-HCL but not in the Tris-HCL-buffered saline soluble fraction. Aβ, amyloid-β; AICD, amyloid precursor protein intracellular domain; APP, amyloid precursor protein; CHO, Chinese hamster ovary; CTF, carboxy-terminal fragment; ND, not determined; NICD, NOTCH intracellular domain; PSEN, presenilin; WT, wild type.

sequentially every three to four amino acids along the α-helical surface of the substrate APP, thereby converting longer Aβ peptides into shorter species [2,50]. In this model, FAD PSEN mutations may cause an overall reduction in proteolytic activity of γ-secretase, and such less efficient, slower mutants may release more Aβ42 molecules from the active center before further trimming of Aβ42 to shorter Aβ peptides. This model also appears to be supported by the fact that the 180 different PSEN1 mutations are scattered over the entire molecule without any apparent hot spots, which is most compatible with the loss-of-function hypothesis.

A small number of knock-in mouse strains for FAD PSEN1 mutations have been created, in which the mutant alleles are expressed under control of the endogenous mouse *PSEN1* promoter [66-69,84]. These studies have either not provided evidence for substantially diminished γ-secretase activity (PSEN1-P264L, PSEN1-R278I) [66,68] or have produced inconclusive results (PSEN1-I213T) [67,84]. The PSEN1-R278I mutation has been particularly well studied in knock-in mice [68] (Table 2). Homozygous knock-in mice for this mutation (R278I/R278I) were embryonic lethal and displayed a phenotype similar to NOTCH knockout mice. In the brain of these mice, accumulation of APP and N-cadherin CTFs was observed, and AICD and NICD fragments were undetectable. This was confirmed in kinetic *in vitro* studies using solubilized membrane preparations from heterozygous (WT/R278I) or homozygous knock-in mice, which showed reduced Aβ and AICD generation from recombinant APP-CTF substrates in a gene-dose-dependent manner. Taken together, these findings indicate a substantial loss of γ-secretase activity of the mutant allele [68]. The decrease in enzymatic activity of the PSEN1-R278I mutant appeared to be particularly severe as other PSEN1 mutations have not caused embryonic lethality in homozygous knock-in mice or were able to rescue the phenotype in *PSEN1*-/- knockout mice [66,67,69,85,86]. Importantly, no developmental defects were observed in heterozygous knock-in mice, and the brain morphology of 3- to 24-month-old mice was indistinguishable from that of WT mice. In brain tissue from heterozygous knock-in mice, no changes in the steady-state levels of APP and N-cadherin CTFs, AICD and NICD were observed, indicating that the γ-secretase-mediated release of intracellular domains is not affected by heterozygous expression of the PSEN1-R278I mutation [68]. A slight decrease in endogenous mouse Aβ40 was detected when brain tissue of 3-month-old heterozygous mice was extracted in guanidine-HCL, but not when these mice were crossed to APP-transgenic mice. Very similar results have been reported for PSEN1-M146V knock-in mice [69]. Interestingly, in APP/PSEN1-R278I double transgenic mice, increased brain levels of Aβ43 were detected that correlated with enhanced amyloid pathology and cognitive deficits, and Aβ43 appeared to induce the formation of Thioflavin T-positive aggregates *in vitro* even more efficiently than Aβ42 [68]. This suggests that Aβ43 might be an overlooked Aβ species that contributes to the formation of neurotoxic Aβ oligomers and plaque pathology. However, it remains to be seen whether other PSEN mutations have any significant effects on the generation and secretion of Aβ43. In summary, *in vitro* studies have provided conclusive evidence that many PSEN mutations cause a substantial loss of γ-secretase activity. However, the results from knock-in mice with heterozygous expression of PSEN mutants indicate that these frequently used cellular assays and, in particular, kinetic *in vitro* assays of PSEN mutants might underestimate the enzymatic activity of γ-secretase in a cellular context where both WT and mutant PSEN alleles contribute to the expressed γ-secretase complexes [66-69]. Importantly, in humans, validated loss-of-function mutations in the genes encoding NOTCH or the γ-secretase subunits PEN-2, PSEN1 and Nicastrin cause skin phenotypes ranging from acne inversa to cutaneous squamous cell carcinomas, as well as chronic myelomonocytic leukaemia [87-89]. In addition, genetic deletion of γ-secretase complex components in mice has demonstrated that a 30% reduction in γ-secretase activity is sufficient to induce a myeloproliferative disease resembling chronic myelomonocytic leukaemia [90]. However, these phenotypes, likely provoked by reduced NOTCH processing and signaling, have never been associated with FAD, further arguing that heterozygous expression of PSEN mutations does not result in a substantial loss of γ-secretase activity [4].

In addition, it has been proposed that FAD PSEN1 mutations impair γ-secretase-independent functions of PSEN proteins in signal transduction, autophagy and calcium homeostasis. The anti-apoptotic phosphatidylinositol 3-kinase-AKT signaling pathway seems to be positively regulated by PSEN proteins. PSEN deficiency or expression of PSEN1 FAD mutants reduced AKT phosphorylation and activity, and increased activity of its downstream target glycogen synthase kinase-3 (GSK-3) [91,92]. In knock-in mice for the PSEN1-I213T mutation, activation of GSK-3β was observed and correlated with increased phosphorylation of its substrate Tau and the formation of intracellular Tau inclusions [93]. Absence of PSEN or expression of PSEN1 FAD mutants has further been demonstrated to impair intracellular protein degradation, caused by a reduced turnover of autophagic vacuoles that fail to become acidified and fuse with lysosomes [60]. Another consistent observation has been that the induced release of calcium from endoplasmic reticulum stores is strongly increased by PSEN FAD

mutants, which might result in deregulation of synaptic transmission and plasticity [61]. A few studies using primary cells from FAD carriers have confirmed that PSEN mutants negatively affect the role of PSEN in autophagic protein degradation and calcium homeostasis [60,61]. With respect to all of these proposed γ-secretase-independent functions, FAD PSEN mutants mimic the phenotype of PSEN deficiency, indicating that the mutants behave as true loss-of-function alleles. While it is certain that PSEN mutants drive amyloidosis in FAD patients, however, the contribution of a potential loss of γ-secretase-independent functions to the clinical phenotype of FAD patients remains to be proven.

Effects of PSEN mutations on small molecules targeting the γ-secretase complex

While investigations of FAD cases have provided invaluable insights into the pathogenesis of AD, patients with FAD further constitute a unique population to conduct treatment or prevention trials with novel pharmaceuticals. Consequently, international consortia aim to recruit FAD patients with PSEN mutations for future clinical trials. In the past, pharmaceutical companies have been cautious to include FAD patients in clinical trials with the argument that novel therapeutics might be less efficacious in these patients because of their specific genetic background or their more aggressive disease course. The described effects of PSEN mutations on small molecules targeting the γ-secretase complex, which constitutes a principal drug target in AD, have provided some support for this caution. Initially, it was described that the efficacy of γ-secretase inhibitors to decrease Aβ production was reduced in cultured cells overexpressing PSEN mutants [94-96]. Similarly, it was demonstrated that PSEN but not APP mutants blocked the effects of γ-secretase modulators (GSMs), which preferentially reduce the amyloidogenic Aβ42 species but spare proteolytic processing of the γ-secretase substrate NOTCH [94,97-100]. The initial studies used GSMs with low *in vitro* potency, which also did not have central nervous system drug properties. Recently, data have been reported for a second generation of potent and clinically relevant GSMs. The overall interpretation of these studies is more complex, with one report showing that PSEN mutants reduced the ability of GSMs to lower Aβ42 irrespective of potency and structural class, and a second study claiming that only few particularly aggressive PSEN mutants rendered cells resistant to these GSMs [97,101]. It is important to note that all these results were obtained in cellular or animal models with overexpression of PSEN mutants. It remains possible, therefore, that the attenuating effect of PSEN mutants might not occur or be negligible when the mutation is expressed in the presence of one WT allele in FAD patients.

A better understanding of presenilin mutations will require improved cellular models

The lack of consensus concerning the effects of PSEN FAD mutations on γ-secretase-dependent and -independent functions and the heterogeneity of results obtained for individual mutations clearly demonstrate that a better understanding of FAD PSEN mutations will require improved cellular models. These models need to account for the heterozygous expression of PSEN mutants in the presence of one WT allele in FAD patients, and they should allow a rigorous comparison of the effects of a larger panel of mutations in a controlled system. Primary fibroblasts or induced pluripotent stem cells derived from human PSEN mutation carriers theoretically provide a suitable cellular model to study the effects of PSEN mutations [102]; however, this approach has serious drawbacks. First, public cell line repositories do not contain primary cells with a sufficient number of different PSEN mutations, and it is at present virtually impossible to acquire cells for specific mutations. Second, a general problem is the lack of genetically matched control cell lines. Commonly, cell lines derived from healthy donors are used as controls, which, because of differences in genetic background and cell derivation, display considerable biological variability. This concern could be addressed in the future through novel methods of genome editing, such as engineered zinc finger nucleases that might allow the generation of isogenic control cell lines [103]. However, these methods are not yet efficient enough to produce adequate numbers of mutant cell lines. Third, even if genetically matched control cells are available, the biological variability between mutant cell lines derived from donors with different PSEN FAD mutations makes them likely unsuitable for stringently controlled biochemical experiments. However, a clear alternative to human patient-derived cell lines are mouse embryonic stem cells, which are more easily amendable to genome editing using site-specific recombinases [104]. Evidently, to establish improved models that faithfully reproduce the genetic and biochemical characteristics of PSEN FAD patients will be laborious and time-consuming, but it is clearly required to overcome the shortcomings of current models based on overexpression of PSEN mutants.

Conclusion

APP and PSEN mutations cause FAD with autosomal-dominant inheritance and early onset disease. FAD is clinically and neuropathologically largely indistinguishable from the sporadic forms of AD, indicating that amyloidosis is a driving force in the etiology of both FAD and sporadic AD. Biochemical studies have shown that APP mutations either shift the generation of Aβ peptides towards the highly amyloidogenic Aβ42 isoform or

enhance the aggregation propensity of the Aβ peptides. No evidence has been found that these mutations impair the physiological function of APP. PSEN mutations also drive amyloidosis in FAD patients through changes in the Aβ42/Aβ40 ratio. In addition, it has been proposed that PSEN mutations could impair other γ-secretase-dependent and -independent functions of PSEN. It is important, however, to note that none of theses phenotypes have been comprehensively replicated in experimental models that bear relevance to the heterozygous genetic background of FAD patients with PSEN mutations. In the few studies that have used primary cells from FAD patients or heterozygous knock-in mice, only single or a small number of PSEN mutations were investigated. It appears premature, therefore, to conclude that loss-of function phenotypes like reduced NOTCH signaling that were reported in overexpression studies with FAD PSEN mutants are relevant to the clinical phenotype of FAD patients, or may even contribute to the pathology of sporadic AD.

Abbreviations

Aβ, amyloid-β; AD, Alzheimer's disease; AICD, amyloid precursor protein intracellular domain; APP, amyloid precursor protein; CAA, cerebral amyloid angiopathy; CTF, carboxy-terminal fragment; FAD, familial Alzheimer's disease; GSM, γ-secretase modulator; HCHWA-D, hereditary cerebral hemorrhage with amyloidosis Dutch-type; NICD, NOTCH intracellular domain; PSEN, presenilin; TMD, transmembrane domain; WT, wild type.

Competing interests

DB is a full-time employee of Merck Serono SA. The authors declare no other competing interests.

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