

Insights into Alzheimer disease pathogenesis from studies in transgenic animal models

Evelin L. Schaeffer,¹ Micheli Figueiró,¹ Wagner F. Gattaz¹

¹Laboratory of Neuroscience (LIM-27), Department and Institute of Psychiatry, Faculty of Medicine, University of São Paulo, Brazil.

Alzheimer disease is the most common cause of dementia among the elderly, accounting for ~60-70% of all cases of dementia. The neuropathological hallmarks of Alzheimer disease are senile plaques (mainly containing β -amyloid peptide derived from amyloid precursor protein) and neurofibrillary tangles (containing hyperphosphorylated Tau protein), along with neuronal loss. At present there is no effective treatment for Alzheimer disease. Given the prevalence and poor prognosis of the disease, the development of animal models has been a research priority to understand pathogenic mechanisms and to test therapeutic strategies. Most cases of Alzheimer disease occur sporadically in people over 65 years old, and are not genetically inherited. Roughly 5% of patients with Alzheimer disease have familial Alzheimer disease—that is, related to a genetic predisposition, including mutations in the amyloid precursor protein, presenilin 1, and presenilin 2 genes. The discovery of genes for familial Alzheimer disease has allowed transgenic models to be generated through the overexpression of the amyloid precursor protein and/or presenilins harboring one or several mutations found in familial Alzheimer disease. Although none of these models fully replicates the human disease, they have provided valuable insights into disease mechanisms as well as opportunities to test therapeutic approaches. This review describes the main transgenic mouse models of Alzheimer disease which have been adopted in Alzheimer disease research, and discusses the insights into Alzheimer disease pathogenesis from studies in such models. In summary, the Alzheimer disease mouse models have been the key to understanding the roles of soluble β -amyloid oligomers in disease pathogenesis, as well as of the relationship between β -amyloid and Tau pathologies.

KEYWORDS: Neurodegenerative disorder; Senile plaques; Neurofibrillary tangles; Neuronal loss; Animal models.

Schaeffer EL, Figueiró M, Gattaz WF. Insights into Alzheimer disease pathogenesis from studies in transgenic animal models. Clinics. 2011;66(S1):45-54.

Received for publication on March 15, 2011; Accepted for publication on March 16, 2011

E-mail: schaeffer@usp.br

Tel.: 55 11 3069-8011

INTRODUCTION

Alzheimer disease (AD), a progressive neurodegenerative disorder, is the most common cause of dementia among the elderly. It accounts for ~60-70% of all dementia cases. Prevalence increases with age from ~1% in the 60-64-year age group, to 24-33% in those aged >85 years.¹ The neuropathological hallmarks of AD are the presence in the brain of extracellular senile plaques and intracellular neurofibrillary tangles, along with neuronal loss. Senile plaques mainly consist of fibrils of 39-42(43) amino acid β -amyloid ($A\beta$) peptide that are surrounded by dystrophic neurites and reactive glial cells. The $A\beta$ peptide itself is derived from the processing of a larger precursor protein known as the amyloid precursor protein (APP).² The dysfunction of APP metabolism and the consequent accumulation of $A\beta$ peptides and their aggregation in the form of senile plaques in the brain parenchyma of individuals with AD, have been considered crucial for neurodegeneration in the disease. This is the so-called “amyloid cascade hypothesis”.^{3,4} However, more recently,

soluble oligomers of $A\beta$ peptide have been correlated with synaptic loss in the brain of AD subjects.⁵⁻⁷ Neurofibrillary tangles contain hyperphosphorylated and aggregated forms of Tau, a microtubule-associated protein that normally promotes the assembly and stability of microtubules in neuronal cells.² Abnormally hyperphosphorylated Tau in AD brain accumulates in neurons into paired helical filaments, which in turn aggregate into neurofibrillary tangles leading to neuronal death.⁸ Therefore, the neuropathological hallmarks of AD induce progressive neuronal dysfunction and degeneration, resulting in severe brain atrophy and decline of memory and other cognitive functions.² Although not a criterion for diagnosis of AD, the deposition of $A\beta$ in the cerebral vasculature, named cerebral amyloid angiopathy (CAA), can be detected in 90% of patients with AD.⁹ However, CAA can occur in the absence of AD pathology and vice versa.¹⁰

Most cases of AD occur sporadically in people over 65 years old, and are not genetically inherited. Roughly 5% of patients with AD have familial Alzheimer disease (FAD), an uncommon form that tends to strike sooner, and is related to a genetic predisposition, including mutations in the APP gene on chromosome 21, presenilin 1 (PS1) gene on chromosome 14, and presenilin 2 (PS2) gene on chromosome 1.¹ The etiology of AD is unclear and at present there is no effective treatment. Given the prevalence and poor prognosis of the disease, the development of animal models has

been a research priority to understand pathogenic mechanisms and to test therapeutic strategies. The discovery of genes for familial forms of AD has allowed transgenic models to be created that reproduce many critical aspects of the disease. Initially, before the discovery of FAD mutations, attempts were made to overexpress wild-type APP in transgenic mice by pronuclear injection. However, none of these efforts produced anything that resembled an A β plaque or any other recognizable AD-type pathology. After the discovery of FAD mutations in APP, a number of groups turned their attention to making AD models based on the overexpression of transgenes containing FAD mutations using a variety of promoters.¹¹ This review describes the main transgenic mouse models of AD which have been adopted in AD research, and discusses the insights into AD pathogenesis from studies in transgenic models.

1. Genetics implicated in Alzheimer disease pathogenesis

Mutations in APP linked to FAD include Dutch (E693Q),¹⁰ London (V717I),¹² Indiana (V717F),¹³ Swedish (K670N/M671L),¹⁴ Florida (I716V),¹⁵ Iowa (D694N),¹⁶ and Arctic (E693G)¹⁷ mutations. To date, more than 160 mutations in PS1 linked to FAD have been discovered (see <http://www.molgen.ua.ac.be/ADMutations>). Mutations in a related gene, now called PS2, were soon linked to FAD as well.¹⁸ Most of FAD mutations cause aberrant APP processing toward the longer, more amyloidogenic A β_{1-42} species.¹⁹ The A β is located partially within the ectodomain (N-terminal portion) and partly within the transmembrane domain (C-terminal portion) of APP. At least three enzymes are responsible for the processing of APP and have been called α -, β - and γ -secretases. The processing pathway by α -secretase, called non-amyloidogenic, cleaves APP within the A β domain in the C-terminal portion of the sequence of this peptide, producing soluble APP α , which has neurotrophic and neuroprotective effects. The processing pathway by β - and γ -secretases, called amyloidogenic, cleaves APP in the N- and C-terminal portions of the A β region, respectively, producing A β peptide. γ -Secretase cleaves APP at

various adjacent sites to form species of A β containing 39 to 43 amino acids.²⁰ Presenilins contribute to the catalytic activity of the γ -secretase complex.¹ Processing of APP by α -, β - and γ -secretases is illustrated in Figure 1.

The Swedish mutation, which is located just outside the N-terminus of the A β domain of APP, favors β -secretase cleavage *in vitro*²¹ and is associated with an increased level and deposition of A β_{1-42} in AD brain.²² The Dutch and Iowa mutations, which are located in the A β domain of APP, accelerate A β_{1-40} fibril formation *in vitro*.^{23,24} The Dutch mutation is associated with cerebrovascular A β deposition—that is, CAA, resulting in cerebral hemorrhages and dementia in patients with AD,¹⁰ whereas the Iowa mutation is associated with severe CAA, widespread neurofibrillary tangles, and unusually extensive distribution of A β_{1-40} in plaques in AD brain.¹⁶ The Arctic mutation, which is also located inside the A β domain, makes APP less available to α -secretase cleavage and increases β -secretase processing of APP thus favoring intracellular A β production *in vitro*.^{25,26} The Arctic mutation is associated with severe CAA in the absence of hemorrhage, abundant parenchymal A β deposits, and neurofibrillary tangles in AD brain.²⁷ The London mutation, which is located in the transmembrane domain of APP, as well as the PS1 and PS2 mutations alter γ -secretase cleavage and increase the A β_{1-42} level and/or the A β_{1-42} /A β_{1-40} ratio *in vitro*.²⁸⁻³⁰ The London mutation is associated with extensive parenchymal A β deposition and abundant senile plaques and neurofibrillary tangles, as well as moderate CAA in AD brain.^{31,32} The Indiana mutation, which is also located in the transmembrane domain of APP, is associated with large number of neurofibrillary tangles and senile plaques, as well as mild CAA in AD brain.³³ The Florida mutation, which is also located in the transmembrane domain of APP, affects γ -secretase cleavage causing an increased A β_{1-42} concentration and A β_{1-42} /A β_{1-40} ratio *in vitro*.^{15,30}

2. Transgenic mouse models of Alzheimer disease

Mouse Models with APP Mutation. Games et al.³⁴ reported the first transgenic AD model, termed **PDAPP mice**, which overexpress a human APP transgene containing the Indiana mutation (V717F) under the control of the platelet-

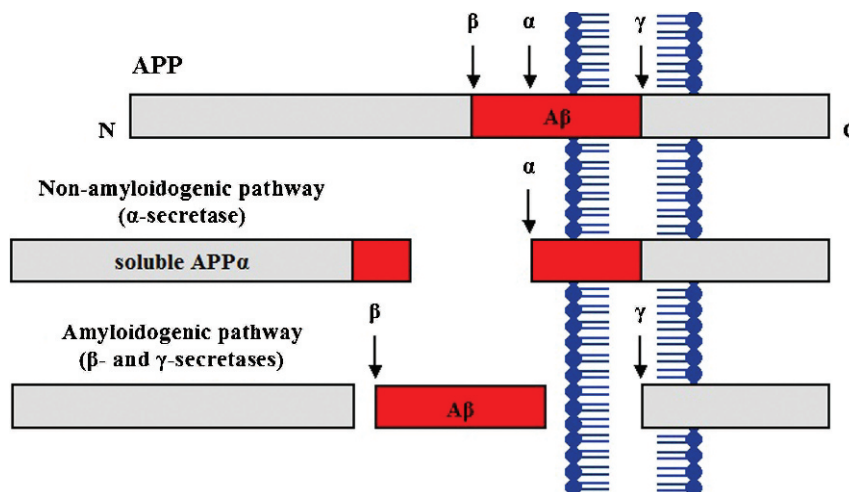


Figure 1 - Schematic diagram illustrating proteolytic cleavage of the amyloid precursor protein (APP). α -Secretase (non-amyloidogenic pathway) cleaves APP within the A β domain to liberate two peptides, including the neuroprotective soluble APP α , whereas β - and γ -secretases (amyloidogenic pathway) act sequentially to cleave APP in the N- and C-terminal portions of the A β region, respectively, producing A β peptide and initiating neurodegenerative activity.

derived growth factor- β promoter. $A\beta_{1-42}$ constituted 27% of the $A\beta$ present in the brain of young PDAPP mice, and this percentage increased to 89% in 12-month-old animals. The mice developed senile plaques that were primarily composed of $A\beta_{1-42}$.³⁵ PDAPP mice showed age-related $A\beta$ deposition in cortical and limbic regions that began at 8 months and progressed to cover 20-50% of the neuropil in the cingulate cortex, entorhinal cortex, and hippocampus of 18-month-old animals. $A\beta$ deposition was associated with dystrophic neurites and extensive gliosis (reactive astrocytes and activated microglia), however, there was no overt neuronal loss in the entorhinal cortex, hippocampal CA1 field, or cingulate cortex through 18 months of age in PDAPP mice.³⁶ Dystrophic neurites immunoreactive for hyperphosphorylated Tau were observed near $A\beta$ plaques after 14 months of age, although no paired helical filaments and neurofibrillary tangles were identified.³⁷ PDAPP mice showed significant and age-dependent synaptic loss, and a rather marked hippocampal atrophy was observed as early as 3 months of age in these mice.³⁸ Young PDAPP mice showed deficits in spatial learning and memory, which worsened with increasing age and $A\beta$ burden.³⁹

Similarly, Hsiao et al.⁴⁰ overexpressed in mice a human APP transgene containing the Swedish mutation (K670N/M671L) driven by a hamster prion protein promoter. These mice, termed **Tg2576 mice**, have been the most widely studied AD transgenic model. Tg2576 mice exhibited age-dependent increase of $A\beta_{1-40}$ and $A\beta_{1-42}$ levels and $A\beta$ deposition, resulting in senile plaques similar to those found in AD. $A\beta$ plaques were first clearly seen by 11-13 months, eventually becoming widespread in cortical and limbic structures.⁴⁰ $A\beta$ deposits were associated with prominent gliosis and neuritic dystrophy, without overt neuronal loss in the hippocampal CA1 field or apparent synapse loss in the hippocampal dentate gyrus.⁴¹ Tg2576 mice exhibited deficits in synaptic plasticity in the hippocampal CA1 field and dentate gyrus, decreased dendritic spine density in the dentate gyrus, and impaired spatial memory and contextual fear conditioning months before significant $A\beta$ deposition, which was detectable at 18 months of age.^{42,43} A decrease in spine density was detected as early as 4 months of age, and synaptic dysfunction and memory impairment were observed by 5 months. Moreover, an increase in the ratio of soluble $A\beta_{1-42}/A\beta_{1-40}$ was first observed at these early ages—that is, at ~4-5 months of age.⁴³ Tg2576 mice also showed increased intraneuronal $A\beta_{1-42}$ accumulation with aging, and this accumulation was associated with abnormal synaptic morphology before $A\beta$ plaque pathology.⁴⁴

Subsequently, many other transgenic lines were developed with approaches similar to those used to develop PDAPP and Tg2576 mice, typically relying on strong promoters to drive overexpression of APP transgenes containing single or multiple FAD mutations. For example, **TgCRND8 mice**, which express multiple human APP mutations—that is, Swedish and Indiana mutations driven by the prion protein promoter, exhibited an aggressive neuropathology with onset of parenchymal $A\beta$ deposition and cognitive deficits as early as 3 months of age, and with dense $A\beta$ plaques and neuritic dystrophy evident from 5 months of age. TgCRND8 mice exhibited an excess of brain $A\beta_{1-42}$ over $A\beta_{1-40}$, and the high-level production of $A\beta_{1-42}$ was associated with spatial learning impairment at 6 months of age. Neurofibrillary tangles and neurodegeneration were absent.⁴⁵ The formation of plaques was concurrent

with the appearance of activated microglia and shortly followed by the clustering of activated astrocytes around plaques at 3.5 months of age in TgCRND8 mice.⁴⁶

Doubly transgenic mice which express human APP with the Swedish mutation driven by the platelet-derived growth factor- β promoter combined with a PS1 mutation (M146L) under the control of the prion protein promoter, termed **APP/PS1 mice**, developed large numbers of fibrillar $A\beta$ deposits in the cerebral cortex and hippocampus that resembled compact $A\beta$ plaques. These mice showed a selective increase in $A\beta_{1-42}$ in their brains and reduced performance in a spatial memory task before substantial $A\beta$ deposition was apparent.⁴⁷ The fibrillar $A\beta$ deposits were associated with dystrophic neurites and activated astrocytes and microglia in APP/PS1 mice.⁴⁸

APP23 mice, which express human APP with only the Swedish mutation driven by a Thy1 promoter, showed neuronal overexpression of APP. At 6 months of age, APP23 mice showed first rare $A\beta$ deposits, which increased with age in size and number and occupied a substantial area of the neocortex and hippocampus in 24-month-old mice. The $A\beta$ plaques were surrounded by gliosis (activated microglia and astrocytes) and dystrophic neurites that were immunoreactive for hyperphosphorylated Tau despite the lack of neurofibrillary tangles.⁴⁹ Determination of plaque-associated $A\beta_{1-42}$ peptides in brain revealed a fivefold increase in APP23 mice at 6 months. In addition, APP23 mice showed an age-dependent decline of spatial memory from the age of 3 months, and locomotor activity and exploratory behavior deficits at 6 months. Spatial memory deficits preceded plaque formation and the increase in plaque-associated $A\beta_{1-42}$ peptides, but correlated negatively with brain soluble $A\beta$ concentration in 3-month-old APP23 mutants.⁵⁰ APP23 mice have often been used to study CAA pathogenesis. Significant deposition of $A\beta$ in the cerebral vasculature—that is, CAA was described in aging APP23 mice. CAA in these mice was associated with local neuronal loss, synaptic loss, microglial activation, and microhemorrhage.^{51,52}

Transgenic mice expressing human APP with the Dutch (E693Q) and Iowa (D694N) mutations combined with the Swedish mutation under the control of the Thy1.2 promoter, termed **Tg-SwDI mice**, developed largely diffuse, $A\beta$ plaque-like deposits in the brain parenchyma starting at 3 months of age with high association with $A\beta$ accumulation in the cerebral microvasculature. $A\beta_{1-40}$ peptides are largely the predominant species that accumulates in these mice.⁵³ Tg-SwDI mice were impaired in the performance of a spatial learning and memory task at 3, 9, and 12 months of age.⁵⁴

APPDutch mice, expressing human APP with only the Dutch mutation regulated by the Thy1 promoter, showed neuronal overexpression of APP and increased ratio of $A\beta_{1-40}/A\beta_{1-42}$ in the brain that resulted in extensive vascular $A\beta$ deposition with essentially no parenchymal deposition.⁵⁵ These researchers also developed a transgenic line that expresses human APP-Dutch mutation crossed with mutant PS1 (G384A), termed **APPDutch/PS1 mice**. These mice, with about half the $A\beta_{1-40}/A\beta_{1-42}$ ratio of APPDutch mice brain, developed parenchymal $A\beta$ plaques with little vascular deposition. By contrast, young transgenic mice harboring human APP with the Arctic mutation (E693G) combined with APP-Swedish and APP-Indiana mutations directed by the platelet-derived growth factor- β promoter, termed **hAPP-Arc mice**, developed prominent parenchymal

A β plaque deposits with little CAA despite a reduced proportion of A β ₁₋₄₂/A β ₁₋₄₀.⁵⁶

Tg-ArcSwe mice with both APP-Swedish and APP-Arctic mutations driven by the Thy1 promoter were developed by two independent groups.^{57,58} Tg-ArcSwe mice exhibited an age-dependent increase in intraneuronal A β accumulation and deficits in spatial memory and contextual fear conditioning, starting at the age of 6 months, before the onset of A β plaque formation as well as CAA.⁵⁷⁻⁵⁹ The cognitive impairments correlated inversely with soluble A β levels in Tg-ArcSwe mice.⁵⁹ Recently, a mouse model expressing human APP with only the Arctic mutation under the control of the Thy1 promoter, termed **APP_{Arctic} mice**, was reported by Rönnbäck et al.⁶⁰ APP_{Arctic} mice showed an age-dependent progression of parenchymal and vascular A β deposition, starting in the subiculum and spreading to the thalamus, and deficits in hippocampus-dependent spatial learning and memory test. In contrast to transgenic models with both the Swedish and Arctic mutations,^{57,58} APP_{Arctic} mice did not show any punctate intraneuronal A β immunoreactivity.⁶⁰

APP transgenic mouse models have been troubled by the difficulty of inducing the characteristic cytoskeletal pathology of AD. For example, in PDAPP mice, phosphorylated Tau sites do accumulate within dystrophic neurites in animals of 14 months of age or older, but there are no paired helical filaments and no neurofibrillary tangle-like lesions.³⁷ Other models have been similar in their lack of any neurofibrillary tangle-like pathology, such as TgCRND8⁴⁵ and APP23 mice.⁴⁹

Mouse Models with Tau Mutation. Transgenic mice that exhibit neurofibrillary tangle-like lesions and A β plaques have been produced by combining FAD mutations with mutant forms of Tau found in a distinct form of dementia known as frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17).⁶¹ Lewis et al.⁶² first crossed Tg2576 mice with a transgenic line known as JNPL3, which expresses P301L mutant Tau associated with FTDP-17, generating a bigenic transgenic model referred to as **TAPP mice**. Singly transgenic JNPL3 mice were known to develop neurofibrillary tangle-like lesions, and TAPP mice exhibited both neurofibrillary tangles and A β plaques. TAPP mice aged 8 months and older displayed more neurofibrillary pathology in limbic regions, most notably the amygdala, than age-matched JNPL3 mice.

Later, Oddo et al.⁶³ generated a triple transgenic model of AD, termed **3xTg-AD mice**, which expressed human APP-Swedish (K670N/M671L) and FTDP-17 Tau (P301L) mutations from exogenous transgenes regulated by the Thy1 promoter combined with a PS1 mutation (M146V) from the endogenous mouse gene. There was a progressive increase in A β formation as a function of age in the 3xTg-AD brain and a particularly pronounced effect on A β ₁₋₄₂ levels. In 3xTg-AD mice, intraneuronal A β accumulation was apparent between 3 and 4 months of age in the neocortex, and at 6 months of age in the hippocampal CA1 field and amygdala. Extracellular A β deposits first became apparent in 6-month-old mice in the frontal cortex and were readily evident by 12 months in other cortical regions and in the hippocampus. A β plaques preceded Tau pathology, which was not evident until about 1 year of age.^{63,64} Tau was conformationally altered and hyperphosphorylated at multiple residues in the brain of 3xTg-AD mice in an age-related manner. Tau-reactive dystrophic neurites were also evident in older

3xTg-AD brain. Functionally, 3xTg-AD mice developed age-dependent synaptic plasticity deficits, but before A β plaque and neurofibrillary tangle pathologies; synaptic dysfunction correlated with the accumulation of intraneuronal A β ₁₋₄₂.⁶³ In addition, these mice manifested earliest retention impairment in spatial memory at 4 months of age that correlated with the accumulation of intraneuronal A β ₁₋₄₂. At 6 months of age, 3xTg-AD mice showed retention deficits in spatial memory and contextual fear conditioning tasks.⁶⁴

Another problem with the AD transgenic mouse models has been the general difficulty of producing neuronal loss. For example, neither PDAPP nor Tg2576 mice, despite having extensive A β deposition, exhibit significant neuronal loss.^{36,41} APP23 mice show only modest losses of pyramidal cells in hippocampal CA1 field (about 15%), losses that are far less than those observed in AD. No quantitative evidence of neuronal loss was observed in the neocortex as a whole.⁶⁵

Mouse Models with Presenilin Mutation. More substantial neuronal loss has been reported in mice expressing multiple APP and PS1 mutations.⁶⁶⁻⁶⁸ One model showing massive neuronal loss is **APP_{SL}/PS1 mice**, which express human APP with the Swedish and London (V717I) mutations driven by the Thy1 promoter and human PS1 with the M146L mutation under the control of the HMG-CoA-reductase promoter. In APP_{SL}/PS1 mice, intraneuronal A β ₁₋₄₀ and A β ₁₋₄₂ stainings preceded A β plaque deposition, which started at 3 months of age. A β was observed in the somatodendritic and axonal compartments of neurons in the subiculum, hippocampal CA1 field, as well as in cortical areas.⁶⁶ The A β ₁₋₄₂/A β ₁₋₄₀ ratio was increased in APP_{SL}/PS1 mice.⁶⁹ A substantial loss (about 30%) of pyramidal neurons in the hippocampal CA1-3 fields was detected in 17-month-old APP_{SL}/PS1 mice. The loss of neurons was observed at sites of A β aggregation and surrounding astrocytes but, most importantly, was also clearly observed in areas of the parenchyma distant from A β plaques.⁷⁰ Furthermore, APP_{SL}/PS1 mice displayed severe age-related synaptic loss within hippocampal dentate gyrus and CA1-3 fields at 17 months of age, even in regions free of extracellular A β deposits.⁶⁹

Another model showing marked neuronal loss expresses human APP-Swedish and APP-London mutations driven by the Thy1 promoter together with two PS1 knock-in (KI) mutations (M233T/L235P) in the murine PS1 gene, and is referred to as **APP/PS1KI mice**. The APP/PS1KI model is so far the model with the most aggressive pathology. These animals showed early extracellular A β deposition at the age of 2.5 months, which was preceded by strong intraneuronal A β accumulation in the hippocampal CA1/2 fields. At 6 months of age, widespread and numerous A β deposits were found within the hippocampal, cortical, and thalamic areas. A β ₁₋₄₂ was the predominant (85%) A β isoform produced in APP/PS1KI mice, and A β ₁₋₄₂ oligomers were highly abundant in the APP/PS1KI brain.⁶⁷ Further pathological features starting at the age of 6 months included severe axonal degeneration, as well as reduced ability to perform working memory and motor tasks.^{71,72} At this time point also synaptic dysfunction and loss became evident in APP/PS1KI brain. In addition, at 6 months of age, a loss of 33% of hippocampal CA1 pyramidal neurons was demonstrated, together with a decreased volume of the CA1 pyramidal cell layer of 30%, and an atrophy of the entire hippocampus of 18%.⁷³ Analysis of the frontal cortex revealed an early loss of

cortical neurons starting at the age of 6 months which correlated with the transient intraneuronal A β accumulation in contrast to extracellular A β plaque pathology.⁷⁴ At 10 months of age, an extensive neuronal loss (>50%) was present in the pyramidal cell layer of hippocampal CA1/2 fields that correlated with strong accumulation of intraneuronal A β but not with extracellular A β deposits in APP/PS1KI mice. A very significant astrogliosis developed in the area of strong intraneuronal A β accumulation and neuronal loss.⁶⁷

Finally, **5xFAD mice** expressing human APP with the Swedish, Florida (I716V) and London mutations together with mutant PS1 (M146L/L286V) regulated by the Thy1 promoter were generated, and robust neuronal loss was observed. 5xFAD mice exhibited dramatically higher levels of A β ₁₋₄₂ than those of A β ₁₋₄₀, and rapidly accumulated massive amounts of cerebral A β ₁₋₄₂ at young ages. A β deposition began at 2 months of age in deep cortical layers and in the subiculum. As mice aged, A β deposits spread to fill much of the cerebral cortex, subiculum, and hippocampus. A β plaques were also observed in the thalamus, brainstem, and olfactory bulb in older mice, but deposits were less numerous in these brain regions. Astrogliosis and microgliosis were proportional to A β ₁₋₄₂ levels and A β deposition in 5xFAD brain and began at approximately the time when plaques initially appeared. Intraneuronal A β ₁₋₄₂ accumulated in 5xFAD brain starting at 1.5 months of age, just before the first appearance of A β deposits at 2 months. Synaptic loss started already at 4 months of age and was significant from 9 months in 5xFAD brain, and large

pyramidal neurons in cortical layer 5 and subiculum were visibly reduced in number at 9 months of age.⁶⁸ 5xFAD mice developed deficits in spatial memory tasks and also exhibited impairments in trace and contextual fear conditioning tests at 4-6 months of age.^{68,75}

Data on the characteristics of the main transgenic mouse models of AD are summarized in Table 1.

3. Insights into Alzheimer disease pathogenesis from studies in transgenic models

Although none of the AD transgenic models fully replicates the human disease, they have suggested new insights into the pathophysiology of A β toxicity, particularly with respect to the effects of different A β species and the possible pathogenic role of A β oligomers.¹¹

In the 1980s it was debated whether A β deposits, and in particular CAA at the cerebral vessel walls, had a central nervous system or a peripheral source.¹¹ Studies in APP23 mice, which developed a similar degree of both A β plaques and CAA, provided the first evidence that a neuronal source of APP/A β is sufficient to induce cerebrovascular A β and associated neurodegeneration.⁵¹ Accordingly, studies in transgenic mice with almost exclusive neuronal central nervous system expression of APP, like APPDutch mice, which develop almost only CAA, strongly suggest that neuronal A β produced in the brain generates cerebrovascular A β neuropathology. In addition, although A β ₁₋₄₂ may be needed as a seed for A β deposition in either compartment (parenchyma and vasculature), studies in APPDutch and

Table 1 - Neuropathological features of the main transgenic mouse models of Alzheimer disease.

Mouse model	Gene (mutation)	Intraneuronal A β	Parenchymal A β plaques	Hyperphosphorylated Tau	Neurofibrillary tangles	Neuronal loss	Synaptic loss	CAA	Primary reference
PDAPP	APP (V717F)	-	Yes	Yes	No	No	Yes	-	Games et al. 1995
Tg2576	APP (K670N/M671L)	Yes	Yes	-	-	No	No	-	Hsiao et al. 1996
TgCRND8	APP (K670N/M671L, V717F)	-	Yes	-	No	No	-	-	Chishti et al. 2001
APP/PS1	APP (K670N/M671L), PS1 (M146L)	-	Yes	-	-	-	-	-	Holcomb et al. 1998
APP23	APP (K670N/M671L)	-	Yes	Yes	No	Little	Yes	Yes	Sturchler-Pierrat et al. 1997
Tg-SwDI	APP (E693Q, D694N)	-	Yes	-	-	-	-	Yes	Davis et al. 2004
APPDutch	APP (E693Q)	-	Little	-	-	-	-	Yes	Herzig et al. 2004
APPDutch/PS1	APP (E693Q), PS1 (G384A)	-	Yes	-	-	-	-	Little	Herzig et al. 2004
hAPP-Arc	APP (E693G, K670N/M671L, V717F)	-	Yes	-	-	-	-	Little	Cheng et al. 2004
Tg-ArcSwe	APP (E693G, K670N/M671L)	Yes	Yes	-	-	-	-	Yes	Lord et al. 2006 Knobloch et al. 2007
APPArc	APP (E693G)	-	Yes	-	-	-	-	Yes	Rönnbäck et al. 2011
TAPP	APP (K670N/M671L), Tau (P301L)	-	Yes	-	Yes	-	-	-	Lewis et al. 2001
3xTg-AD	APP (K670N/M671L), Tau (P301L), PS1 (M146V)	Yes	Yes	Yes	Yes	-	No	-	Oddo et al. 2003
APP _{SL} /PS1	APP (K670N/M671L, V717I), PS1 (M146L)	Yes	Yes	-	-	Yes	Yes	-	Wirths et al. 2002
APP/PS1KI	APP (K670N/M671L, V717I), PS1 (M233T/L235P)	Yes	Yes	-	-	Yes	Yes	-	Casas et al. 2004
5xFAD	APP (K670N/M671L, I716V, V717I), PS1 (M146L/L286V)	Yes	Yes	-	-	Yes	Yes	-	Oakley et al. 2006

CAA = cerebral amyloid angiopathy; Dash (-) = not reported.

APPDutch/PS1 mice suggest that A β_{1-40} promotes vascular deposition, whereas A β_{1-42} shifts deposition toward parenchymal A β .⁵⁵ Moreover, studies in hAPP-Arc mice, with APP-Arctic mutation (E693G) combined with APP-Swedish and APP-Indiana mutations, suggest that some property of the APP E693G mutation, besides its effect on the A β_{1-40} /A β_{1-42} ratio, may also influence parenchymal deposition versus vascular deposition.⁵⁶ Therefore, the existing AD transgenic mouse models have shown considerable utility in deciphering the pathobiology of CAA.

Analyses of the brain of APP transgenic mouse models in which large amounts of A β have accumulated in plaques but no neurodegeneration has developed, such as PDAPP,^{35,36} Tg2576,^{40,41} TgCRND8,⁴⁵ and APP23⁶⁵ mice, provide no or very sparse support for the well-established amyloid cascade hypothesis. This hypothesis supports the idea that increased A β production and extracellular accumulation in plaques leads to neurotoxicity, resulting in widespread neuronal loss and dementia.⁷⁶ Some reasons for this have been discussed. Perhaps the neurotoxicity is sparse in APP mouse models because murine neurons might be devoid of the downstream pathways necessary for A β to induce toxicity, such as the processes leading to Tau aggregation and neurofibrillary tangle formation in AD brain.¹¹ Interestingly, subsequent to the original amyloid hypothesis, it became clear that A β plaque counts correlate relatively poorly with the level of cognitive decline in AD and that the number of neurofibrillary tangles correlates more strongly with the degree of dementia.⁷⁷ Perhaps only certain species of A β (A β_{1-40} , A β_{1-42} , or truncated A β) are neurotoxic, and by using mutations linked to familial AD we poorly replicate the processes of A β production and aggregation in sporadic AD brain.¹¹ Curiously, truncated A β peptides were demonstrated in AD brain more than 10 years ago,^{78,79} but the observations were partially ignored. Today it is well established that only a fraction of A β in *postmortem* AD brain is full-length A β_{1-40} or A β_{1-42} ; N-terminally truncated variants of A β (A β_{3-42} and A β_{11-42}) are prevalent in senile plaques of AD brain.^{80,81} Unlike the classical amyloid cascade hypothesis, it was subsequently shown that soluble oligomers of A β_{1-42} and not plaque-associated A β correlate best with cognitive dysfunction in AD.^{6,82}

There is now a great interest in identifying which A β species (A β_{1-40} , A β_{1-42} , or truncated A β) and form (oligomers or deposits) would be responsible for neurotoxicity, and in understanding the relationship between A β and Tau pathologies.¹¹ APP transgenic mice have provided strong evidence for the toxicity of soluble A β oligomers *in vivo* by showing that many pathological and functional changes in mice occur before the appearance of A β plaque pathology. For example, studies in PDAPP mice demonstrated that loss of volume in the hippocampus, predominantly localized to the dentate gyrus, was present in 100-day-old mice well before A β deposition in plaques.⁸³ In addition, loss in total dendritic length was evident in the dentate gyrus of 90-day-old PDAPP mice well before A β accumulation occurs.⁸⁴ Tg2576 mice exhibited increased ratio of soluble A β_{1-42} /A β_{1-40} , deficits in synaptic plasticity in the hippocampal CA1 field and dentate gyrus, loss of dendritic spines in the dentate gyrus, and impaired spatial and contextual memory months before significant A β deposition.^{42,43} In APP23 mice, spatial memory deficits preceded plaque formation and the increase in plaque-associated A β_{1-42} peptides, but correlated negatively with soluble A β concentration.⁵⁰ Tg-ArcSwe mice exhibited robust deficits in spatial memory and contextual fear

conditioning before the onset of A β deposition,⁵⁷⁻⁵⁹ and the cognitive impairments correlated inversely with soluble A β levels.⁵⁹ 3xTg-AD mice developed age-dependent synaptic plasticity deficits and spatial memory impairment before A β plaque and neurofibrillary tangle pathologies but instead in correlation with soluble A β_{1-42} .^{63,64} Finally, APP/PS1 mice, which exhibit large numbers of compact A β plaques in the cerebral cortex and hippocampus, showed a selective increase in A β_{1-42} in their brains and reduced performance in a spatial memory task in the period preceding overt A β deposition.⁴⁷ These studies are consistent with the more critical role of A β_{1-42} in the pathogenesis of AD and suggest a neurotoxic effect of soluble forms of A β .

Since the discovery that truncated A β_{3-42} represents a major species in senile plaques of AD brain,^{80,81} this peptide has received considerable attention. In comparison with A β_{1-42} , A β_{3-42} has stronger aggregation propensity and increased toxicity *in vitro*.⁸⁵⁻⁸⁷ Recently, a new transgenic mouse model (TBA2) was generated,⁸⁸ which expressed only truncated A β_{3-42} in neurons without any of the other A β peptides, and it was demonstrated for the first time that this peptide is neurotoxic *in vivo*, inducing neuronal loss and concomitant neurological deficits characterized by loss of motor coordination and ataxia.

In the past, A β has been regarded as acting extracellularly, whereas recent evidence points to toxic effects of A β in intracellular compartments. First reports showing that A β is initially deposited in neurons before occurring in the extracellular space date back roughly 20 years.^{89,90} More recently, it has been shown that neurons in AD-vulnerable regions accumulate A β_{1-42} and it has been further suggested that this accumulation precedes extracellular A β deposition and neurofibrillary tangle formation.⁹¹ Consecutively, a variety of reports has been published demonstrating A β in neurons of AD brain.⁹²⁻⁹⁵ Curiously, soluble A β oligomers, which have been suggested as the most toxic species, are formed, preferentially, intracellularly within neuronal processes and synapses rather than extracellularly.^{96,97} In all transgenic mouse models in which marked neuronal loss has been so far reported, this was preceded by considerable amounts of intraneuronal A β peptides.⁹⁸ For example, in APP/PS1KI mice, which developed severe learning deficits correlating with CA1 field neuronal loss and hippocampal atrophy, increased intraneuronal A β_{1-42} and not plaque-associated A β coincided well with neuronal loss; the intraneuronal N-truncated A β_{3-42} species was also increased, however, the dominant species was A β_{1-42} in the APP/PS1KI model.^{67,73} In agreement with this study, investigations in TBA2 mice showed for the first time that intraneuronal A β_{3-42} accumulation is sufficient for triggering neuronal death and inducing an associated neurological phenotype. Although the TBA2 model lacks important AD-typical neuropathological features like tangles and hippocampal degeneration, it clearly demonstrated that intraneuronal A β_{3-42} is neurotoxic *in vivo*.⁸⁸ Intraneuronal A β_{1-42} accumulation has also been reported in several transgenic mouse models with no overt neuronal loss, including Tg2576,⁴⁴ 3xTg-AD,⁶³ and 5xFAD.⁶⁸ These studies indicate that intraneuronal soluble A β is a pathological feature of AD that has long been neglected and is turning out to be the key factor leading to neuronal loss in the disease before the extracellular A β deposition.

Loss of neuronal synaptic density and synapse number represents another invariant feature of AD that appears to precede overt neuronal degeneration.^{99,100} Notably, it has been

shown that the loss of synaptic terminals correlates better with cognitive decline than plaque and tangle load or neuronal loss, leading to the concept that losing synapses is one of the key events leading to cognitive dysfunction in AD.^{37,101-104} There is accumulating evidence from AD transgenic mice that intraneuronal A β ₁₋₄₂ triggers not only early neuronal loss but also synaptic deficits. For example, Tg2576 mice showed increased intraneuronal A β ₁₋₄₂ accumulation with aging, and this accumulation was associated with abnormal synaptic morphology before A β plaque pathology.⁴⁴ 3xTg-AD mice developed age-dependent synaptic plasticity deficits, but before A β plaque and neurofibrillary tangle pathologies; synaptic dysfunction correlated with the accumulation of intraneuronal A β ₁₋₄₂.⁶³ Intraneuronal A β ₁₋₄₂ accumulated in 5xFAD brain starting at 1.5 months of age, just before the first appearance of A β deposits at 2 months. Synaptic loss started already at 4 months of age and was significant from 9 months in 5xFAD brain, whereas local neuronal loss first became apparent at 9 months of age.⁶⁸ The development of the APP_{SL}/PS1 mice, which exhibit intraneuronal A β ₁₋₄₂ accumulation, offered for the first time the possibility to address the question of whether alterations in synaptic integrity precede neuronal loss in a transgenic animal model of AD, and the data indicated that loss of neurons was of limited impact on age-related synaptic loss and that at least part of synaptic loss seen in regions free of A β deposits was due to elevated levels of soluble A β oligomers.⁶⁹

Regarding the interaction between A β and Tau pathologies, although A β plaque development is almost certainly driven by the APP and PS1 FAD mutations, whereas the tangle-like pathology is driven by the Tau mutations, it does appear that such mutations interact, as suggested by studies in transgenic mouse models with Tau mutation. For example, bigenic TAPP mice (expressing K670N/M671L mutant APP and P301L mutant Tau) have enhanced neurofibrillary pathology in limbic regions, most notably the amygdala, in comparison with transgenic JNPL3 animals (expressing singly P301L mutant Tau), suggesting that the formation of Tau inclusions might be influenced by increasing the level of APP or A β peptides.⁶² Additionally, intracerebral injections of anti-A β antibodies into the hippocampus of 3xTg-AD mice not only reduced A β accumulation but also resulted in clearance of early-stage, but not late-stage, hyperphosphorylated Tau aggregates. Whereas A β deposits were cleared within 3 days, the Tau lesions required a slightly longer time and were not reduced until 5 days after injection. Thus, A β was cleared first, followed by the clearance of Tau localized in the somatodendritic compartment. Conversely, by 30 days after injection, A β deposits reemerged, although the Tau pathology was not apparent at this time point.¹⁰⁵ These studies thus show that modulating A β affects Tau pathology and suggest that Tau pathology may be downstream of A β generation.

CONCLUSION

To study AD, a variety of transgenic mouse models has been generated through the overexpression of the APP and/or the presenilins harboring one or several mutations found in familial AD.^{34,40,45,49,53,55-58,60,62,63,66-68} Although none of the AD transgenic mice models reproduces the human condition exactly, the ability to study similar pathological processes in living animals has provided valuable insights into disease mechanisms and opportunities to test therapeutic approaches.¹¹ The AD mouse models have been key to understanding the roles of soluble A β oligomers in disease

pathogenesis, as well as of the relationship between A β and Tau pathologies. Data obtained from the comparison of different AD mouse lines indicate that the onset and the severity of the A β deposits are directly linked to the level of soluble A β ₁₋₄₂ peptide.^{42,43,47,58,59,63,64,83,84} There is accumulating evidence from AD transgenic mice that intraneuronal A β ₁₋₄₂ triggers early neuronal loss as well as synaptic deficits.^{63,67-69,73} Studies in a transgenic animal model of AD that exhibits marked neuronal and synaptic loss indicate that alterations in synaptic integrity precede neuronal loss,⁶⁹ which is in accordance with the hypothesis that synaptic loss is one of the earliest events in AD pathogenesis.^{37,101-104} Furthermore, evidence from AD transgenic mouse models supports the notion that A β may directly or indirectly interact with Tau to accelerate neurofibrillary tangle formation.^{62,105} Finally, the AD transgenic models may allow to define and evaluate potential drug targets and to develop therapeutic strategies that might interfere or delay the onset of AD.¹⁰⁶

REFERENCES

1. Philipson O, Lord A, Gumucio A, O'Callaghan P, Lannfelt L, Nilsson LN. Animal models of amyloid-beta-related pathologies in Alzheimer's disease. *FEBS J*. 2010;277:1389-409, doi: 10.1111/j.1742-4658.2010.07564.x.
2. Selkoe DJ. Aging, amyloid, and Alzheimer's disease: a perspective in honor of Carl Cotman. *Neurochem Res*. 2003;28:1705-13, doi: 10.1023/A:1026065122854.
3. Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. *Science*. 1992;256:184-5, doi: 10.1126/science.1566067.
4. Estus S, Tucker HM, van Rooyen C, Wright S, Brigham EF, Wogulis M, et al. Aggregated amyloid-beta protein induces cortical neuronal apoptosis and concomitant "apoptotic" pattern of gene induction. *J Neurosci*. 1997;17:7736-45.
5. Lue LF, Kuo YM, Roher AE, Brachova L, Shen Y, Sue L, et al. Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. *Am J Pathol*. 1999;155:853-62, doi: 10.1016/S0002-9440(10)65184-X.
6. McLean CA, Cherny RA, Fraser FW, Fuller SJ, Smith MJ, Beyreuther K, et al. Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. *Ann Neurol*. 1999;46:860-6, doi: 10.1002/1531-8249(199912)46:6<860::AID-ANA8>3.0.CO;2-M.
7. Wang J, Dickson DW, Trojanowski JQ, Lee VM. The levels of soluble versus insoluble brain Abeta distinguish Alzheimer's disease from normal and pathologic aging. *Exp Neurol*. 1999;158:328-37, doi: 10.1006/exnr.1999.7085.
8. Iqbal K, Grundke-Iqbal I. Discoveries of tau, abnormally hyperphosphorylated tau and others of neurofibrillary degeneration: a personal historical perspective. *J Alzheimers Dis*. 2006;9:219-42.
9. Vinters HV. Cerebral amyloid angiopathy. A critical review. *Stroke*. 1987;18:311-24.
10. Levy E, Carman MD, Fernandez-Madrid IJ, Power MD, Lieberburg I, van Duinen SG, et al. Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch type. *Science*. 1990;248:1124-6, doi: 10.1126/science.2111584.
11. Elder GA, Gama Sosa MA, De Gasperi R. Transgenic mouse models of Alzheimer's disease. *Mt Sinai J Med*. 2010;77:69-81, doi: 10.1002/msj.20159.
12. Goate A, Chartier-Harlin MC, Mullan M, Brown J, Crawford F, Fidani L, et al. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature*. 1991;349:704-6, doi: 10.1038/349704a0.
13. Murrell J, Farlow M, Ghetti B, Benson MD. A mutation in the amyloid precursor protein associated with hereditary Alzheimer's disease. *Science*. 1991;254:97-9, doi: 10.1126/science.1925564.
14. Mullan M, Crawford F, Axelman K, Houlden H, Lilius L, Winblad B, et al. A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of beta-amyloid. *Nat Genet*. 1992;1:345-7, doi: 10.1038/ng0892-345.
15. Eckman CB, Mehta ND, Crook R, Perez-tur J, Prihar G, Pfeiffer E, et al. A new pathogenic mutation in the APP gene (I716V) increases the relative proportion of A beta 42(43). *Hum Mol Genet*. 1997;6:2087-9, doi: 10.1093/hmg/6.12.2087.
16. Grabowski TJ, Cho HS, Vonsattel JP, Rebeck GW, Greenberg SM. Novel amyloid precursor protein mutation in an Iowa family with dementia and severe cerebral amyloid angiopathy. *Ann Neurol*. 2001;49:697-705, doi: 10.1002/ana.1009.

17. Nilsberth C, Westlind-Danielsson A, Eckman CB, Condron MM, Axelman K, Forsell C, et al. The 'Arctic' APP mutation (E693G) causes Alzheimer's disease by enhanced Abeta protofibril formation. *Nat Neurosci.* 2001;4:887-93, doi: 10.1038/nn0901-887.
18. Ertekin-Taner N. Genetics of Alzheimer's disease: a centennial review. *Neurol Clin.* 2007;25:611-67, doi: 10.1016/j.ncl.2007.03.009.
19. Kowalska A. Genetic aspects of amyloid beta-protein fibrillogenesis in Alzheimer's disease. *Folia Neuropathol.* 2004;42:235-7.
20. Numan J, Small DH. Regulation of APP cleavage by alpha-, beta- and gamma-secretases. *FEBS Lett.* 2000;483:6-10, doi: 10.1016/S0014-5793(00)02076-7.
21. Perez RG, Squazzo SL, Koo EH. Enhanced release of amyloid beta-protein from codon 670/671 "Swedish" mutant beta-amyloid precursor protein occurs in both secretory and endocytic pathways. *J Biol Chem.* 1996;271:9100-7.
22. Mann DM, Iwatsubo T, Ihara Y, Cairns NJ, Lantos PL, Bogdanovic N, et al. Predominant deposition of amyloid-beta 42(43) in plaques in cases of Alzheimer's disease and hereditary cerebral hemorrhage associated with mutations in the amyloid precursor protein gene. *Am J Pathol.* 1996;148:1257-66.
23. Wisniewski T, Ghiso J, Frangione B. Peptides homologous to the amyloid protein of Alzheimer's disease containing a glutamine for glutamic acid substitution have accelerated amyloid fibril formation. *Biochem Biophys Res Commun.* 1991;179:1247-54, doi: 10.1016/0006-291X(91)91706-I.
24. Van Nostrand WE, Melchor JP, Cho HS, Greenberg SM, Rebeck GW. Pathogenic effects of D23N Iowa mutant amyloid beta -protein. *J Biol Chem.* 2001;276:32860-6, doi: 10.1074/jbc.M104135200.
25. Steinh C, Nilsberth C, Hammarback J, Engvall B, Näslund J, Lannfelt L. The Arctic mutation interferes with processing of the amyloid precursor protein. *Neuroreport.* 2002;13:1857-60, doi: 10.1097/00001756-200210280-00005.
26. Sahlín C, Lord A, Magnusson K, Englund H, Almeida CG, Greengard P, et al. The Arctic Alzheimer mutation favors intracellular amyloid-beta production by making amyloid precursor protein less available to alpha-secretase. *J Neurochem.* 2007;101:854-62, doi: 10.1111/j.1471-4159.2006.04443.x.
27. Basun H, Bogdanovic N, Ingelsson M, Almkvist O, Näslund J, Axelman K, et al. Clinical and neuropathological features of the arctic APP gene mutation causing early-onset Alzheimer disease. *Arch Neurol.* 2008;65:499-505, doi: 10.1001/archneur.65.4.499.
28. Borchelt DR, Thinakaran G, Eckman CB, Lee MK, Davenport F, Ratovitsky T, et al. Familial Alzheimer's disease-linked presenilin 1 variants elevate Abeta1-42/1-40 ratio in vitro and in vivo. *Neuron.* 1996;17:1005-13, doi: 10.1016/S0896-6273(00)80230-5.
29. Xia W, Zhang J, Kholodenko D, Citron M, Podlisy MB, Teplow DB, et al. Enhanced production and oligomerization of the 42-residue amyloid beta-protein by Chinese hamster ovary cells stably expressing mutant presenilins. *J Biol Chem.* 1997;272:7977-82, doi: 10.1074/jbc.272.12.7977.
30. De Jonghe C, Esselens C, Kumar-Singh S, Craessaerts K, Serneels S, Checler F, et al. Pathogenic APP mutations near the gamma-secretase cleavage site differentially affect Abeta secretion and APP C-terminal fragment stability. *Hum Mol Genet.* 2001;10:1665-71, doi: 10.1093/hmg/10.16.1665.
31. Lantos PL, Luthert PJ, Hanger D, Anderton BH, Mullan M, Rossor M. Familial Alzheimer's disease with the amyloid precursor protein position 717 mutation and sporadic Alzheimer's disease have the same cytoskeletal pathology. *Neurosci Lett.* 1992;137:221-4, doi: 10.1016/0304-3940(92)90408-Y.
32. Mullan M, Tsuji S, Miki T, Katsuya T, Naruse S, Kaneko K, et al. Clinical comparison of Alzheimer's disease in pedigrees with the codon 717 Val->Ile mutation in the amyloid precursor protein gene. *Neurobiol Aging.* 1993;14:407-19, doi: 10.1016/0197-4580(93)90099-W.
33. Ghetti B, Murrell J, Benson MD, Farlow MR. Spectrum of amyloid beta-protein immunoreactivity in hereditary Alzheimer disease with a guanine to thymine missense change at position 1924 of the APP gene. *Brain Res.* 1992;571:133-9, doi: 10.1016/0006-8993(92)90519-F.
34. Games D, Adams D, Alessandrini R, Barbour R, Berthelette P, Blackwell C, et al. Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. *Nature.* 1995;373:523-7, doi: 10.1038/373523a0.
35. Johnson-Wood K, Lee M, Motter R, Hu K, Gordon G, Barbour R, et al. Amyloid precursor protein processing and A beta42 deposition in a transgenic mouse model of Alzheimer disease. *Proc Natl Acad Sci USA.* 1997;94:1550-5, doi: 10.1073/pnas.94.4.1550.
36. Irizarry MC, Soriano F, McNamara M, Page KJ, Schenk D, Games D, et al. Abeta deposition is associated with neuropil changes, but not with overt neuronal loss in the human amyloid precursor protein V717F (PDAPP) transgenic mouse. *J Neurosci.* 1997;17:7053-9.
37. Masliah E, Sisk A, Mallory M, Games D. Neurofibrillary pathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. *J Neuropathol Exp Neurol.* 2001;60:357-68.
38. Dodart JC, Mathis C, Saura J, Bales KR, Paul SM, Ungerer A. Neuroanatomical abnormalities in behaviorally characterized APP(V717F) transgenic mice. *Neurobiol Dis.* 2000;7:71-85, doi: 10.1006/nbdi.1999.0278.
39. Chen G, Chen KS, Knox J, Inglis J, Bernard A, Martin SJ, et al. A learning deficit related to age and beta-amyloid plaques in a mouse model of Alzheimer's disease. *Nature.* 2000;408:975-9, doi: 10.1038/35046031.
40. Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, et al. Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. *Science.* 1996;274:99-102, doi: 10.1126/science.274.5284.99.
41. Irizarry MC, McNamara M, Fedorchak K, Hsiao K, Hyman BT. APPSw transgenic mice develop age-related A beta deposits and neuropil abnormalities, but no neuronal loss in CA1. *J Neuropathol Exp Neurol.* 1997;56:965-73, doi: 10.1097/00005072-199709000-00002.
42. Chapman PF, White GL, Jones MW, Cooper-Blacketer D, Marshall VJ, Irizarry M, et al. Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice. *Nat Neurosci.* 1999;2:271-6, doi: 10.1038/6374.
43. Jacobsen JS, Wu CC, Redwine JM, Comery TA, Arias R, Bowlby M, et al. Early-onset behavioral and synaptic deficits in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci USA.* 2006;103:5161-6, doi: 10.1073/pnas.0600948103.
44. Takahashi RH, Milner TA, Li F, Nam EE, Edgar MA, Yamaguchi H, et al. Intra-neuronal Alzheimer abeta42 accumulates in multivesicular bodies and is associated with synaptic pathology. *Am J Pathol.* 2002;161:1869-79, doi: 10.1016/S0002-9440(10)64463-X.
45. Chishti MA, Yang DS, Janus C, Phinney AL, Horne P, Pearson J, et al. Early-onset amyloid deposition and cognitive deficits in transgenic mice expressing a double mutant form of amyloid precursor protein 695. *J Biol Chem.* 2001;276:21562-70, doi: 10.1074/jbc.M100710200.
46. Duda S, Krzywkowski P, Paquette J, Morissette C, Lacombe D, Tremblay P, et al. Inflammation occurs early during the Abeta deposition process in TgCRND8 mice. *Neurobiol Aging.* 2004;25:861-71, doi: 10.1016/j.neurobiolaging.2003.08.008.
47. Holcomb L, Gordon MN, McGowan E, Yu X, Benkovic S, Jantzen P, et al. Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nat Med.* 1998;4:97-100, doi: 10.1038/nm0198-097.
48. Gordon MN, Holcomb LA, Jantzen PT, DiCarlo G, Wilcock D, Boyett KW, et al. Time course of the development of Alzheimer-like pathology in the doubly transgenic PS1+APP mouse. *Exp Neurol.* 2002;173:183-95, doi: 10.1006/exnr.2001.7754.
49. Sturchler-Pierrat C, Abramowski D, Duke M, Wiederhold KH, Mistl C, Rothacher S, et al. Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. *Proc Natl Acad Sci USA.* 1997;94:13287-92, doi: 10.1073/pnas.94.24.13287.
50. Van Dam D, D'Hooge R, Staufenbiel M, Van Ginneken C, Van Meir F, De Deyn PP. Age-dependent cognitive decline in the APP23 model precedes amyloid deposition. *Eur J Neurosci.* 2003;17:388-96, doi: 10.1046/j.1460-9568.2003.02444.x.
51. Calhoun ME, Burgermeister P, Phinney AL, Stalder M, Tolnay M, Wiederhold KH, et al. Neuronal overexpression of mutant amyloid precursor protein results in prominent deposition of cerebrovascular amyloid. *Proc Natl Acad Sci USA.* 1999;96:14088-93, doi: 10.1073/pnas.96.24.14088.
52. Winkler DT, Bondolfi L, Herzig MC, Jann L, Calhoun ME, Wiederhold KH, et al. Spontaneous hemorrhagic stroke in a mouse model of cerebral amyloid angiopathy. *J Neurosci.* 2001;21:1619-27.
53. Davis J, Xu F, Deane R, Romanov G, Previti ML, Zeigler K, et al. Early-onset and robust cerebral microvascular accumulation of amyloid beta-protein in transgenic mice expressing low levels of a vasculotropic Dutch/Iowa mutant form of amyloid beta-protein precursor. *J Biol Chem.* 2004;279:20296-306, doi: 10.1074/jbc.M312946200.
54. Xu F, Grande AM, Robinson JK, Previti ML, Vasek M, Davis J, et al. Early-onset subicular microvascular amyloid and neuroinflammation correlate with behavioral deficits in vasculotropic mutant amyloid beta-protein precursor transgenic mice. *Neuroscience.* 2007;146:98-107, doi: 10.1016/j.neuroscience.2007.01.043.
55. Herzig MC, Winkler DT, Burgermeister P, Pfeifer M, Kohler E, Schmidt SD, et al. Abeta is targeted to the vasculature in a mouse model of hereditary cerebral hemorrhage with amyloidosis. *Nat Neurosci.* 2004;7:954-60, doi: 10.1038/nn1302.
56. Cheng IH, Palop JJ, Esposito LA, Bien-Ly N, Yan F, Mucke L. Aggressive amyloidosis in mice expressing human amyloid peptides with the Arctic mutation. *Nat Med.* 2004;10:1190-2, doi: 10.1038/nm1123.
57. Lord A, Kalimo H, Eckman C, Zhang XQ, Lannfelt L, Nilsson LN. The Arctic Alzheimer mutation facilitates early intraneuronal Abeta aggregation and senile plaque formation in transgenic mice. *Neurobiol Aging.* 2006;27:67-77, doi: 10.1016/j.neurobiolaging.2004.12.007.

58. Knobloch M, Konietzko U, Krebs DC, Nitsch RM. Intracellular Abeta and cognitive deficits precede beta-amyloid deposition in transgenic arcAbeta mice. *Neurobiol Aging*. 2007;28:1297-306, doi: 10.1016/j.neurobiolaging.2006.06.019.
59. Lord A, Englund H, Söderberg L, Tucker S, Clausen F, Hillered L, et al. Amyloid-beta protofibril levels correlate with spatial learning in Arctic Alzheimer's disease transgenic mice. *FEBS J*. 2009;276:995-1006, doi: 10.1111/j.1742-4658.2008.06836.x.
60. Rönnbäck A, Zhu S, Dillner K, Aoki M, Lilius L, Näslund J, et al. Progressive neuropathology and cognitive decline in a single Arctic APP transgenic mouse model. *Neurobiol Aging*. 2011;32:280-92, doi: 10.1016/j.neurobiolaging.2009.02.021.
61. Haugarvoll K, Wszolek ZK, Hutton M. The genetics of frontotemporal dementia. *Neurol Clin*. 2007;25:697-715, doi: 10.1016/j.ncl.2007.03.002.
62. Lewis J, Dickson DW, Lin WL, Chisholm L, Corral A, Jones G, et al. Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. *Science*. 2001;293:1487-91, doi: 10.1126/science.1058189.
63. Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kaye R, et al. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. *Neuron*. 2003;39:409-21, doi: 10.1016/S0896-6273(03)00434-3.
64. Billings LM, Oddo S, Green KN, McGaugh JL, LaFerla FM. Intraneuronal Abeta causes the onset of early Alzheimer's disease-related cognitive deficits in transgenic mice. *Neuron*. 2005;45:675-88, doi: 10.1016/j.neuron.2005.01.040.
65. Calhoun ME, Kurth D, Phinney AL, Long JM, Hengemihle J, Mouton PR, et al. Hippocampal neuron and synaptophysin-positive bouton number in aging C57BL/6 mice. *Neurobiol Aging*. 1998;19:599-606, doi: 10.1016/S0197-4580(98)00098-0.
66. Wirths O, Multhaup G, Czech C, Feldmann N, Blanchard V, Tremp G, et al. Intraneuronal APP/A beta trafficking and plaque formation in beta-amyloid precursor protein and presenilin-1 transgenic mice. *Brain Pathol*. 2002;12:275-86, doi: 10.1111/j.1750-3639.2002.tb00442.x.
67. Casas C, Sergeant N, Itier JM, Blanchard V, Wirths O, van der Kolk N, et al. Massive CA1/2 neuronal loss with intraneuronal and N-terminal truncated Abeta42 accumulation in a novel Alzheimer transgenic model. *Am J Pathol*. 2004;165:1289-300, doi: 10.1016/S0002-9440(10)63388-3.
68. Oakley H, Cole SL, Logan S, Maus E, Shao P, Craft J, et al. Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. *J Neurosci*. 2006;26:10129-40, doi: 10.1523/JNEUROSCI.1202-06.2006.
69. Rutten BP, Van der Kolk NM, Schafer S, van Zandvoort MA, Bayer TA, Steinbusch HW, et al. Age-related loss of synaptophysin immunoreactive presynaptic boutons within the hippocampus of APP751SL, PS1M146L, and APP751SL/PS1M146L transgenic mice. *Am J Pathol*. 2005;167:161-73, doi: 10.1016/S0002-9440(10)62963-X.
70. Schmitz C, Rutten BP, Pielen A, Schäfer S, Wirths O, Tremp G, et al. Hippocampal neuron loss exceeds amyloid plaque load in a transgenic mouse model of Alzheimer's disease. *Am J Pathol*. 2004;164:1495-502, doi: 10.1016/S0002-9440(10)63235-X.
71. Wirths O, Weis J, Kaye R, Saido TC, Bayer TA. Age-dependent axonal degeneration in an Alzheimer mouse model. *Neurobiol Aging*. 2007;28:1689-99, doi: 10.1016/j.neurobiolaging.2006.07.021.
72. Wirths O, Breyhan H, Schäfer S, Roth C, Bayer TA. Deficits in working memory and motor performance in the APP/PS1ki mouse model for Alzheimer's disease. *Neurobiol Aging*. 2008;29:891-901, doi: 10.1016/j.neurobiolaging.2006.12.004.
73. Breyhan H, Wirths O, Duan K, Marcello A, Rettig J, Bayer TA. APP/PS1KI bigenic mice develop early synaptic deficits and hippocampus atrophy. *Acta Neuropathol*. 2009;117:677-85, doi: 10.1007/s00401-009-0539-7.
74. Christensen DZ, Kraus SL, Flohr A, Cotel MC, Wirths O, Bayer TA. Transient intraneuronal A beta rather than extracellular plaque pathology correlates with neuron loss in the frontal cortex of APP/PS1KI mice. *Acta Neuropathol*. 2008;116:647-55, doi: 10.1007/s00401-008-0451-6.
75. Ohno M, Cole SL, Yasvoina M, Zhao J, Citron M, Berry R, et al. BACE1 gene deletion prevents neuron loss and memory deficits in 5XFAD APP/PS1 transgenic mice. *Neurobiol Dis*. 2007;26:134-45, doi: 10.1016/j.nbd.2006.12.008.
76. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science*. 2002;297:353-6, doi: 10.1126/science.1072994.
77. Arriagada PV, Marzloff K, Hyman BT. Distribution of Alzheimer-type pathologic changes in nondemented elderly individuals matches the pattern in Alzheimer's disease. *Neurology*. 1992;42:1681-8.
78. Roher AE, Lowenson JD, Clarke S, Wolkow C, Wang R, Cotter RJ, et al. Structural alterations in the peptide backbone of beta-amyloid core protein may account for its deposition and stability in Alzheimer's disease. *J Biol Chem*. 1993;268:3072-83.
79. Saido TC, Iwatsubo T, Mann DM, Shimada H, Ihara Y, Kawashima S. Dominant and differential deposition of distinct beta-amyloid peptide species, A beta N3(pE), in senile plaques. *Neuron*. 1995;14:457-66, doi: 10.1016/0896-6273(95)90301-1.
80. Russo C, Saido TC, DeBusk LM, Tabaton M, Gambetti P, Teller JK. Heterogeneity of water-soluble amyloid beta-peptide in Alzheimer's disease and Down's syndrome brains. *FEBS Lett*. 1997;409:411-6, doi: 10.1016/S0014-5793(97)00564-4.
81. Russo C, Salis S, Dolcini V, Venezia V, Song XH, Teller JK, et al. Amino-terminal modification and tyrosine phosphorylation of [corrected] carboxy-terminal fragments of the amyloid precursor protein in Alzheimer's disease and Down's syndrome brain. *Neurobiol Dis*. 2001;8:173-180, doi: 10.1006/ndbi.2000.0357.
82. Näslund J, Haroutunian V, Mohs R, Davis KL, Davies P, Greengard P, et al. Correlation between elevated levels of amyloid beta-peptide in the brain and cognitive decline. *JAMA*. 2000;283:1571-7, doi: 10.1001/jama.283.12.1571.
83. Redwine JM, Kosofsky B, Jacobs RE, Games D, Reilly JF, Morrison JH, et al. Dentate gyrus volume is reduced before onset of plaque formation in PDAPP mice: a magnetic resonance microscopy and stereologic analysis. *Proc Natl Acad Sci USA*. 2003;100:1381-6, doi: 10.1073/pnas.242746599.
84. Wu CC, Chawla F, Games D, Rydel RE, Freedman S, Schenk D, et al. Selective vulnerability of dentate granule cells prior to amyloid deposition in PDAPP mice: digital morphometric analyses. *Proc Natl Acad Sci USA*. 2004;101:7141-6, doi: 10.1073/pnas.0402147101.
85. He W, Barrow CJ. The A beta 3-pyroglutanyl and 11-pyroglutanyl peptides found in senile plaque have greater beta-sheet forming and aggregation propensities in vitro than full-length A beta. *Biochemistry*. 1999;38:10871-7, doi: 10.1021/bi990563r.
86. Russo C, Violani E, Salis S, Venezia V, Dolcini V, Damonte G, et al. Pyroglutamate-modified amyloid beta-peptides--AbetaN3(pE)--strongly affect cultured neuron and astrocyte survival. *J Neurochem*. 2002;82:1480-9, doi: 10.1046/j.1471-4159.2002.01107.x.
87. Schilling S, Lauber T, Schaupp M, Manhart S, Scheel E, Böhm G, et al. On the seeding and oligomerization of pGlu-amyloid peptides (in vitro). *Biochemistry*. 2006;45:12393-9, doi: 10.1021/bi0612667.
88. Wirths O, Breyhan H, Cynis H, Schilling S, Demuth HU, Bayer TA. Intraneuronal pyroglutamate-Abeta 3-42 triggers neurodegeneration and lethal neurological deficits in a transgenic mouse model. *Acta Neuropathol*. 2009;118:487-96, doi: 10.1007/s00401-009-0557-5.
89. Masters CL, Multhaup G, Simms G, Pottgiesser J, Martins RN, Beyreuther K. Neuronal origin of a cerebral amyloid: neurofibrillary tangles of Alzheimer's disease contain the same protein as the amyloid of plaque cores and blood vessels. *EMBO J*. 1985;4:2757-63.
90. Grundke-Iqbal I, Iqbal K, George L, Tung YC, Kim KS, Wisniewski HM. Amyloid protein and neurofibrillary tangles coexist in the same neuron in Alzheimer disease. *Proc Natl Acad Sci USA*. 1989;86:2853-7, doi: 10.1073/pnas.86.8.2853.
91. Gouras GK, Tsai J, Naslund J, Vincent B, Edgar M, Checler F, et al. Intraneuronal Abeta42 accumulation in human brain. *Am J Pathol*. 2000;156:15-20, doi: 10.1016/S0002-9440(10)64700-1.
92. Mochizuki A, Tamaoka A, Shimohata A, Komatsuzaki Y, Shoji S. Abeta42-positive non-pyramidal neurons around amyloid plaques in Alzheimer's disease. *Lancet*. 2000;355:42-3, doi: 10.1016/S0140-6736(99)04937-5.
93. D'Andrea MR, Nagele RG, Wang HY, Lee DH. Consistent immunohistochemical detection of intracellular beta-amyloid42 in pyramidal neurons of Alzheimer's disease entorhinal cortex. *Neurosci Lett*. 2002;333:163-6, doi: 10.1016/S0304-3940(02)00875-3.
94. D'Andrea MR, Nagele RG, Wang HY, Peterson PA, Lee DH. Evidence that neurones accumulating amyloid can undergo lysis to form amyloid plaques in Alzheimer's disease. *Histopathology*. 2001;38:120-34, doi: 10.1046/j.1365-2559.2001.01082.x.
95. Fernández-Vizcarra P, Fernández AP, Castro-Blanco S, Serrano J, Bentura ML, Martínez-Murillo R, et al. Intra- and extracellular Abeta and PHF in clinically evaluated cases of Alzheimer's disease. *Histol Histopathol*. 2004;19:823-44.
96. Walsh DM, Tseng BP, Rydel RE, Podlisny MB, Selkoe DJ. The oligomerization of amyloid beta-protein begins intracellularly in cells derived from human brain. *Biochemistry*. 2000;39:10831-9, doi: 10.1021/bi001048s.
97. Takahashi RH, Almeida CG, Kearney PF, Yu F, Lin MT, Milner TA, et al. Oligomerization of Alzheimer's beta-amyloid within processes and synapses of cultured neurons and brain. *J Neurosci*. 2004;24:3592-9, doi: 10.1523/JNEUROSCI.5167-03.2004.
98. Bayer TA, Wirths O. Intracellular accumulation of amyloid-Beta - a predictor for synaptic dysfunction and neuron loss in Alzheimer's disease. *Front Aging Neurosci*. 2010;2:8.
99. DeKosky ST, Scheff SW. Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. *Ann Neurol*. 1990;27:457-64, doi: 10.1002/ana.410270502.
100. Scheff SW, Scott SA, DeKosky ST. Quantitation of synaptic density in the septal nuclei of young and aged Fischer 344 rats. *Neurobiol Aging*. 1991;12:3-12, doi: 10.1016/0197-4580(91)90032-F.

101. Flood DG, Coleman PD. Hippocampal plasticity in normal aging and decreased plasticity in Alzheimer's disease. *Prog Brain Res.* 1990;83:435-43, doi: 10.1016/S0079-6123(08)61267-4.
102. Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, et al. Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Ann Neurol.* 1991;30:572-80, doi: 10.1002/ana.410300410.
103. Dickson DW, Crystal HA, Bevona C, Honer W, Vincent I, Davies P. Correlations of synaptic and pathological markers with cognition of the elderly. *Neurobiol Aging.* 1995;16:285-98; discussion 298-304, doi: 10.1016/0197-4580(95)00013-5.
104. Sze CI, Troncoso JC, Kawas C, Mouton P, Price DL, Martin LJ. Loss of the presynaptic vesicle protein synaptophysin in hippocampus correlates with cognitive decline in Alzheimer disease. *J Neuropathol Exp Neurol.* 1997;56:933-44, doi: 10.1097/00005072-199708000-00011.
105. Oddo S, Billings L, Kesslak JP, Cribbs DH, LaFerla FM. Abeta immunotherapy leads to clearance of early, but not late, hyperphosphorylated tau aggregates via the proteasome. *Neuron.* 2004;43:321-32, doi: 10.1016/j.neuron.2004.07.003.
106. Wisniewski T, Boutajangout A. Immunotherapeutic approaches for Alzheimer's disease in transgenic mouse models. *Brain Struct Funct.* 2010;214:201-18, doi: 10.1007/s00429-009-0236-2.