Insights into Alzheimer disease pathogenesis from studies in transgenic animal models

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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

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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 (Aβ) peptide that are surrounded by dystrophic neurites and reactive glial cells. The Aβ 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 Aβ 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 Aβ 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 Aβ 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 Aβ 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 $A\beta_{1-42}$ species.[19] The Aβ 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 Aβ domain in the C-terminal portion of the sequence of this peptide, producing soluble APPα, 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 Aβ region, respectively, producing Aβ peptide. γ-Secretase cleaves APP at various adjacent sites to form species of Aβ 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 Aβ domain of APP, favors β-secretase cleavage *in vitro*[21] and is associated with an increased level and deposition of $A\beta_{1-42}$ in AD brain.[22] The Dutch and Iowa mutations, which are located in the Aβ domain of APP, accelerate $A\beta_{1-40}$ fibril formation *in vitro*.[23,24] The Dutch mutation is associated with cerebrovascular Aβ 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 $A\beta_{1-40}$ in plaques in AD brain.[16] The Arctic mutation, which is also located inside the Aβ domain, makes APP less available to α-secretase cleavage and increases β-secretase processing of APP thus favoring intracellular Aβ production *in vitro*.[25,26] The Arctic mutation is associated with severe CAA in the absence of hemorrhage, abundant parenchymal Aβ 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 $A\beta_{1-42}$ level and/or the $A\beta_{1-42}/A\beta_{1-40}$ ratio *in vitro*.[28-30] The London mutation is associated with extensive parenchymal Aβ 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 $A\beta_{1-42}$ concentration and $A\beta_{1-42}/A\beta_{1-40}$ 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-

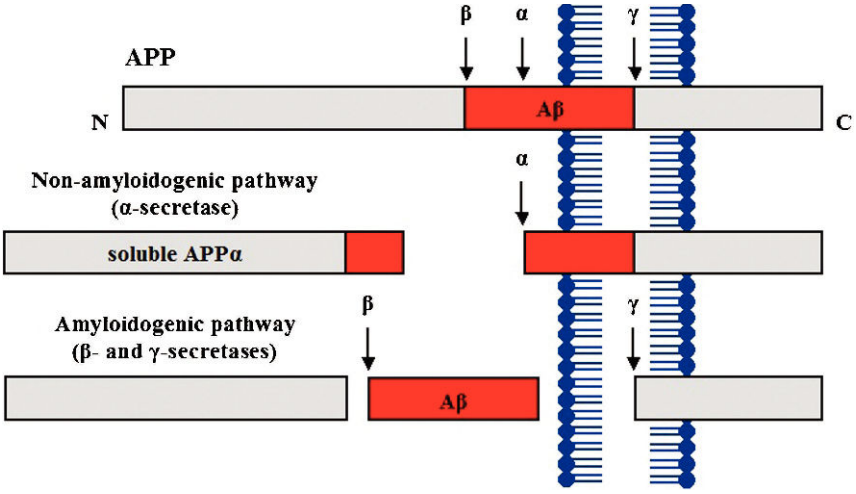

Figure 1 - Schematic diagram illustrating proteolytic cleavage of the amyloid precursor protein (APP). α-Secretase (non-amyloidogenic pathway) cleaves APP within the Aβ domain to liberate two peptides, including the neuroprotective soluble APPα, whereas β- and γ-secretases (amyloidogenic pathway) act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

derived growth factor-β promoter. $A\beta_{1-42}$ constituted 27% of the Aβ 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 Aβ 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. Aβ 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 Aβ 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 Aβ 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 Aβ deposition, resulting in senile plaques similar to those found in AD. Aβ plaques were first clearly seen by 11-13 months, eventually becoming widespread in cortical and limbic structures.[40] Aβ 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 Aβ 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 Aβ 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 Aβ deposition and cognitive deficits as early as 3 months of age, and with dense Aβ 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 Aβ deposits in the cerebral cortex and hippocampus that resembled compact Aβ 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 Aβ deposition was apparent.[47] The fibrillar Aβ 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 Aβ 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 Aβ 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 Aβ concentration in 3-month-old APP23 mutants.[50] APP23 mice have often been used to study CAA pathogenesis. Significant deposition of Aβ 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, Aβ plaque-like deposits in the brain parenchyma starting at 3 months of age with high association with Aβ 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 Aβ 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 Aβ 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

Aβ plaque deposits with little CAA despite a reduced proportion of $A\beta_{1-42}/A\beta_{1-40}$.[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 Aβ accumulation and deficits in spatial memory and contextual fear conditioning, starting at the age of 6 months, before the onset of Aβ plaque formation as well as CAA.[57-59] The cognitive impairments correlated inversely with soluble Aβ 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 **APPArc mice**, was reported by Rönnbäck et al.[60] APPArc mice showed an age-dependent progression of parenchymal and vascular Aβ 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] APPArc mice did not show any punctate intraneuronal Aβ 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 TgCRND8[45] and APP23 mice.[49]

Mouse Models with Tau Mutation. Transgenic mice that exhibit neurofibrillary tangle-like lesions and Aβ 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 Aβ 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 Aβ formation as a function of age in the 3xTg-AD brain and a particularly pronounced effect on $A\beta_{1-42}$ levels. In 3xTg-AD mice, intraneuronal Aβ 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 Aβ 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. Aβ 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 Aβ plaque and neurofibrillary tangle pathologies; synaptic dysfunction correlated with the accumulation of intraneuronal $A\beta_{1-42}$.[63] In addition, these mice manifested earliest retention impairment in spatial memory at 4 months of age that correlated with the accumulation of intraneuronal $A\beta_{1-42}$. 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 Aβ 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 $A\beta_{1-40}$ and $A\beta_{1-42}$ stainings preceded Aβ plaque deposition, which started at 3 months of age. Aβ was observed in the somatodendritic and axonal compartments of neurons in the subiculum, hippocampal CA1 field, as well as in cortical areas.[66] The $A\beta_{1-42}/A\beta_{1-40}$ 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 Aβ aggregation and surrounding astrocytes but, most importantly, was also clearly observed in areas of the parenchyma distant from Aβ 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 Aβ 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 Aβ deposition at the age of 2.5 months, which was preceded by strong intraneuronal Aβ accumulation in the hippocampal CA1/2 fields. At 6 months of age, widespread and numerous Aβ deposits were found within the hippocampal, cortical, and thalamic areas. $A\beta_{1-42}$ was the predominant (85%) Aβ isovariant produced in APP/PS1KI mice, and $A\beta_{1-42}$ 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 Aβ accumulation in contrast to extracellular Aβ 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 Aβ but not with extracellular Aβ deposits in APP/PS1KI mice. A very significant astrogliosis developed in the area of strong intraneuronal Aβ 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 $A\beta_{1-42}$ than those of $A\beta_{1-40}$, and rapidly accumulated massive amounts of cerebral $A\beta_{1-42}$ at young ages. Aβ deposition began at 2 months of age in deep cortical layers and in the subiculum. As mice aged, Aβ deposits spread to fill much of the cerebral cortex, subiculum, and hippocampus. Aβ 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 $A\beta_{1-42}$ levels and Aβ deposition in 5xFAD brain and began at approximately the time when plaques initially appeared. Intraneuronal $A\beta_{1-42}$ accumulated in 5xFAD brain starting at 1.5 months of age, just before the first appearance of Aβ 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 Aβ toxicity, particularly with respect to the effects of different Aβ species and the possible pathogenic role of Aβ oligomers.[11]

In the 1980s it was debated whether Aβ 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 Aβ plaques and CAA, provided the first evidence that a neuronal source of APP/Aβ is sufficient to induce cerebrovascular Aβ 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 Aβ produced in the brain generates cerebrovascular Aβ neuropathology. In addition, although $A\beta_{1-42}$ may be needed as a seed for Aβ 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 Aβ	Parenchymal Aβ 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 $A\beta_{1-40}$ promotes vascular deposition, whereas $A\beta_{1-42}$ shifts deposition toward parenchymal Aβ.[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 $A\beta_{1-40}/A\beta_{1-42}$ 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 Aβ have accumulated in plaques but no neurodegeneration has developed, such as PDAPP,[35,36] Tg2576,[40,41] TgCRND8,[45] and APP23[65] mice, provide no or very sparse support for the well-established amyloid cascade hypothesis. This hypothesis supports the idea that increased Aβ 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 Aβ 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 Aβ 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 Aβ ($A\beta_{1-40}$, $A\beta_{1-42}$, or truncated Aβ) are neurotoxic, and by using mutations linked to familial AD we poorly replicate the processes of Aβ production and aggregation in sporadic AD brain.[11] Curiously, truncated Aβ 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 Aβ in *postmortem* AD brain is full-length $A\beta_{1-40}$ or $A\beta_{1-42}$; N-terminally truncated variants of Aβ ($A\beta_{3-42}$ and $A\beta_{11-42}$) are prevalent in senile plaques of AD brain.[80,81] Unlike the classical amyloid cascade hypothesis, it was subsequently shown that soluble oligomers of $A\beta_{1-42}$ and not plaque-associated Aβ correlate best with cognitive dysfunction in AD.[6,82]

There is now a great interest in identifying which Aβ species ($A\beta_{1-40}$, $A\beta_{1-42}$, or truncated Aβ) and form (oligomers or deposits) would be responsible for neurotoxicity, and in understanding the relationship between Aβ and Tau pathologies.[11] APP transgenic mice have provided strong evidence for the toxicity of soluble Aβ oligomers *in vivo* by showing that many pathological and functional changes in mice occur before the appearance of Aβ 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 Aβ 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 Aβ accumulation occurs.[84] Tg2576 mice exhibited increased ratio of soluble $A\beta_{1-42}/A\beta_{1-40}$, 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 Aβ deposition.[42,43] In APP23 mice, spatial memory deficits preceded plaque formation and the increase in plaque-associated $A\beta_{1-42}$ peptides, but correlated negatively with soluble Aβ concentration.[50] Tg-ArcSwe mice exhibited robust deficits in spatial memory and contextual fear conditioning before the onset of Aβ deposition,[57-59] and the cognitive impairments correlated inversely with soluble Aβ levels.[59] 3xTg-AD mice developed age-dependent synaptic plasticity deficits and spatial memory impairment before Aβ plaque and neurofibrillary tangle pathologies but instead in correlation with soluble $A\beta_{1-42}$.[63,64] Finally, APP/PS1 mice, which exhibit large numbers of compact Aβ plaques in the cerebral cortex and hippocampus, showed a selective increase in $A\beta_{1-42}$ in their brains and reduced performance in a spatial memory task in the period preceding overt Aβ deposition.[47] These studies are consistent with the more critical role of $A\beta_{1-42}$ in the pathogenesis of AD and suggest a neurotoxic effect of soluble forms of Aβ.

Since the discovery that truncated $A\beta_{3-42}$ represents a major species in senile plaques of AD brain,[80,81] this peptide has received considerable attention. In comparison with $A\beta_{1-42}$, $A\beta_{3-42}$ 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 $A\beta_{3-42}$ in neurons without any of the other Aβ 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, Aβ has been regarded as acting extracellularly, whereas recent evidence points to toxic effects of Aβ in intracellular compartments. First reports showing that Aβ 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 $A\beta_{1-42}$ and it has been further suggested that this accumulation precedes extracellular Aβ deposition and neurofibrillary tangle formation.[91] Consecutively, a variety of reports has been published demonstrating Aβ in neurons of AD brain.[92-95] Curiously, soluble Aβ 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 Aβ peptides.[98] For example, in APP/PS1KI mice, which developed severe learning deficits correlating with CA1 field neuronal loss and hippocampal atrophy, increased intraneuronal $A\beta_{1-42}$ and not plaque-associated Aβ coincided well with neuronal loss; the intraneuronal N-truncated $A\beta_{3-42}$ species was also increased, however, the dominant species was $A\beta_{1-42}$ in the APP/PS1KI model.[67,73] In agreement with this study, investigations in TBA2 mice showed for the first time that intraneuronal $A\beta_{3-42}$ 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 $A\beta_{3-42}$ is neurotoxic *in vivo*.[88] Intraneuronal $A\beta_{1-42}$ 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 Aβ 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 Aβ 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 $A\beta_{1-42}$ triggers not only early neuronal loss but also synaptic deficits. For example, Tg2576 mice showed increased intraneuronal $A\beta_{1-42}$ accumulation with aging, and this accumulation was associated with abnormal synaptic morphology before Aβ plaque pathology.[44] 3xTg-AD mice developed age-dependent synaptic plasticity deficits, but before Aβ plaque and neurofibrillary tangle pathologies; synaptic dysfunction correlated with the accumulation of intraneuronal $A\beta_{1-42}$.[63] Intraneuronal $A\beta_{1-42}$ accumulated in 5xFAD brain starting at 1.5 months of age, just before the first appearance of Aβ 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 $A\beta_{1-42}$ 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 Aβ deposits was due to elevated levels of soluble Aβ oligomers.[69]

Regarding the interaction between Aβ and Tau pathologies, although Aβ 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 Aβ peptides.[62] Additionally, intracerebral injections of anti-Aβ antibodies into the hippocampus of 3xTg-AD mice not only reduced Aβ accumulation but also resulted in clearance of early-stage, but not late-stage, hyperphosphorylated Tau aggregates. Whereas Aβ deposits were cleared within 3 days, the Tau lesions required a slightly longer time and were not reduced until 5 days after injection. Thus, Aβ was cleared first, followed by the clearance of Tau localized in the somatodendritic compartment. Conversely, by 30 days after injection, Aβ deposits reemerged, although the Tau pathology was not apparent at this time point.[105] These studies thus show that modulating Aβ affects Tau pathology and suggest that Tau pathology may be downstream of Aβ 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 Aβ oligomers in disease pathogenesis, as well as of the relationship between Aβ and Tau pathologies. Data obtained from the comparison of different AD mouse lines indicate that the onset and the severity of the Aβ deposits are directly linked to the level of soluble $A\beta_{1-42}$ peptide.[42,43,47,58,59,63,64,83,84] There is accumulating evidence from AD transgenic mice that intraneuronal $A\beta_{1-42}$ 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 Aβ 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]

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