

Mouse Models of Alzheimer's Disease: A Quest for Plaques and Tangles

James A. Richardson and Dennis K. Burns

Abstract

Many genetically altered mice have been designed to help understand the role of specific gene mutations in the pathogenesis of Alzheimer's disease (AD) based on the realization that specific mutations in the genes for amyloid precursor protein—the presenilins and tau—are associated with early-onset familial AD or, in the case of tau mutations, other neurodegenerative diseases with neurofibrillary tangles. However, attempts to reproduce the neuropathology of AD in the mouse have been frustrating. Transgenic designs emphasizing amyloid precursor protein produced mice that develop amyloid plaques, but neurodegeneration and neurofibrillary tangles failed to form. Strategies emphasizing tau resulted in increased phosphorylation of tau and tangle formation, although amyloid plaques were absent. Nevertheless, crossing transgenic animals expressing mutated tau and amyloid precursor protein has produced a mouse that closely recapitulates the neuropathology of AD. A review of the various murine models, their role in understanding the pathogenesis of AD and their use in testing therapeutic regimens, is provided.

Key Words: Alzheimer's disease; amyloid plaque; neurofibrillary tangle; tau; transgenic mice

Introduction

Alzheimer's disease (AD¹) is the most common cause of dementia in the elderly and affects an estimated 4 million people in the United States alone. The majority of cases of AD are sporadic, although in roughly 10% of cases, there is a family history of dementia. These cases of familial AD (FAD¹) have provided important insights

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¹Abbreviations used in this article: A β , amyloid β protein; AD, Alzheimer's disease; ApoE, apolipoprotein E; APP, amyloid precursor protein; BACE1, β -site APP-cleaving enzyme 1; BACE2, β -site APP-cleaving enzyme 2; FAD, familial Alzheimer's disease; FTDP-17, frontotemporal dementia with Parkinsonism linked to chromosome 17; NFT, neurofibrillary tangles; PDGF- β , platelet-derived growth factor β ; PS1, presenilin 1; PS2, presenilin 2.

into the pathogenesis of AD in recent years. In several pedigrees of early-onset FAD, point mutations in several genes have been identified. Mutations in the genes encoding amyloid- β precursor protein (APP¹), presenilin 1 (PS1¹), and presenilin 2 (PS2¹) are associated with early-onset autosomal dominant AD. In addition, AD is associated with the accumulation of hyperphosphorylated tau within degenerating neurons. The rationale for creating the various transgenic mouse models of AD has been to explore the function of these molecules in vivo and to establish a mouse model with histopathological and clinical features that parallel AD in humans.

Morphological Changes in AD

The brain is typically atrophic in AD, although it may be grossly normal, in the early stages of the disease particularly. When present, atrophy is typically most pronounced in the frontal, temporal, and parietal lobes. Examination of the cut surface reveals symmetrical dilation of the ventricular system in most cases, reflecting a generalized loss of brain parenchyma.

Microscopically, AD is characterized by the presence of filamentous protein aggregates (termed neurofibrillary tangles) within the cytoplasm of neurons in the neocortex, hippocampus, basal forebrain, and some areas of the brainstem (Figure 1A). Specifics regarding the postmortem diagnosis of AD are outlined in a consensus recommendation from the National Institute on Aging (Hyman and Trojanowski 1997). The neurofibrillary tangles are composed of course neuritic processes containing insoluble protein-rich helical filaments, the major component of which is hyperphosphorylated tau protein. Filamentous processes (termed neuropil threads), likely representing altered dendritic processes, also accumulate in the neuropil in AD in a distribution similar to that of the neurofibrillary tangles; and they contain tau and other abnormal cytoskeletal proteins similar to those present in neurofibrillary tangles. Additional accumulations of tau-rich paired helical filaments occur within distal neuronal cell processes (neurites) to form so-called senile plaques (Figure 1B), which appear as aggregates of coarse tortuous neurites in the neuropil of the cerebral cortex and hippocampus. The senile plaques also contain a central core composed of amyloid β protein (A β ¹). A β deposits are usually present within the walls of leptomeningeal as well as smaller parenchymal vessels, a change referred to as amyloid angiopathy.

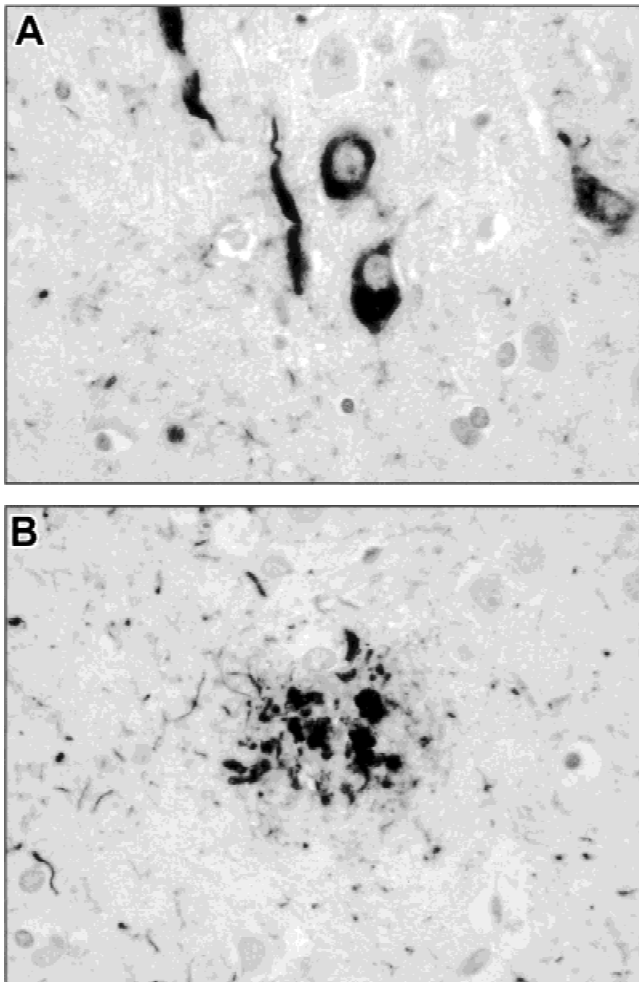


Figure 1 (A) Section of hippocampus, from an elderly patient with Alzheimer's disease, stained with antibody to hyperphosphorylated tau protein, demonstrates densely staining neurofibrillary tangles within neuronal cell bodies. (B) Immunohistochemical stain from a case similar to (A) demonstrates a typical neuritic plaque, composed of clusters of swollen neuritic cell processes laden with hyperphosphorylated tau protein. Diaminobenzidine-labeled section $\times 400$.

The simple presence of neurofibrillary tangles and/or senile plaques is not, by itself, entirely specific for AD inasmuch as identical structures are also frequently present in the brains of cognitively normal elderly individuals. It is, rather, the density and widespread distribution of these changes in the cerebral neocortex that leads to the diagnosis of AD.

Molecular Components in the Pathogenesis of AD

Amyloid precursor protein, PS1, PS2, and tau, are believed to be strongly associated with the incidence of AD in humans. Consequently, the mouse models of AD were created

by manipulating these molecules. A review of the pertinent molecular components follows.

One of the principal components of senile plaques in brains affected with AD is amyloid β protein. $A\beta$ is secreted constitutively by normal cells in culture and is detected as circulating peptide in the plasma and cerebrospinal fluid of healthy humans and other mammals. $A\beta$ is derived by endoproteolytic cleavage of APP, which has a large extracellular domain, a single small transmembrane region, and a small cytoplasmic tail. Mutations in the APP gene encoded on chromosome 21 account for a small fraction of the cases of FAD.

APP occurs in several different isoforms, which arise from alternative splicing of a single gene. The shortest of the major isoforms (695 amino acids) is expressed almost exclusively in neurons, whereas the other two common forms (751 and 770 amino acids, respectively) are expressed both in neuronal and non-neuronal cells. Mice null for APP are viable, but they exhibit reactive gliosis and have a decreased locomotor activity and forelimb grip strength (Zheng et al. 1995).

APP has a short half-life and is metabolized by proteases (termed secretases) along two pathways, one nonamyloidogenic and one amyloidogenic (Figure 2). α -Secretase initiates the nonamyloidogenic pathway by cleaving APP between extracellular residues 687 and 688 within the $A\beta$ domain, releasing the large soluble ectodomain, βAPP_s . This initial cleavage is followed by endoproteolytic cleavage of the C-terminal fragment within its transmembrane domain by an enzyme activity (termed γ secretase) to produce a short fragment (termed p3), which is released con-

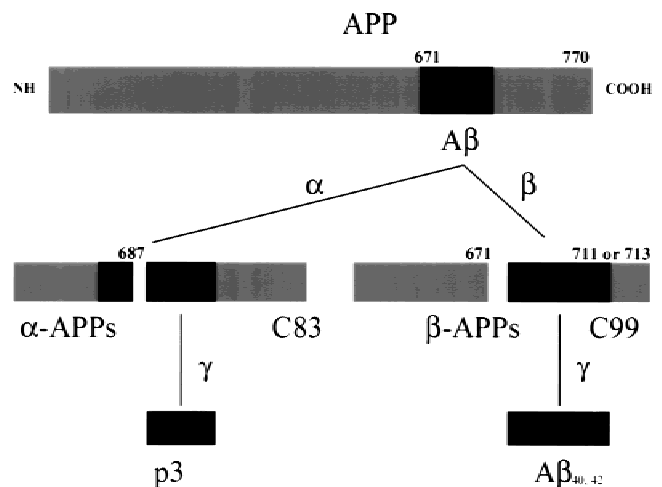


Figure 2 Schematic of amyloid precursor protein (APP) processing. The $A\beta$ portion of the molecule is shown in black. When APP is processed by α secretase, it yields a secreted fragment αAPP_s and the membrane-bound C83. Further cleavage by γ secretase produces p3. Alternatively, cleavage of APP by β secretase produces the membrane-bound fragment C99, which is cleaved by γ secretase to form $\alpha\beta_{40,42}$.

stitutively by APP-expressing cells during normal metabolism. β -Secretase initiates the amyloidogenic pathway by cleavage of APP after amino acid 671, creating a 99-residue membrane-retained C-terminal fragment having residue 1 of $A\beta$ as its N terminus. The truncated N-terminal fragment of APP, βAPP_S , is released. The C-terminal fragment is then cleaved by γ -secretase to produce $A\beta$.

Of key importance in the pathway described above is the site of the β -secretase cleavage. If the cleavage is between amino acids 712 and 713, $A\beta$ is produced. However, if it is cut after amino acid 714, the larger $A\beta_{40}$ is formed. The majority of the secreted $A\beta$ peptides are the short soluble $A\beta_{40}$ variety. However, approximately 10% of the secreted $A\beta$ peptides are the more insoluble $A\beta_{42}$, which readily aggregate to form extracellular fibrils. This heterogeneity of the $A\beta$ fragment is important inasmuch as $A\beta_{42}$ is first to appear in the diffuse plaques of Down syndrome patients whereas $A\beta_{40}$ is not detected until decades later (Selkoe 1998).

Not all of the C-terminal fragments produced by α - and β -secretases are cleaved by γ -secretase to $A\beta$ and p3. Alternatively, proteolytic pathways can fully degrade the C-terminal fragments in endosomes and lysosomes (Selkoe 1998). The identity of the various secretases involved in the cellular processing of APP has been elusive. Recently two β -secretases have been identified: β -site APP-cleaving enzyme ($BACE^1$) 1 and BACE2 (Sinha et al. 1999; Vassar et al. 1999; Yan et al. 1999). BACE1 is highly expressed in the brain and is the major β -secretase for the generation of $A\beta$ peptides by neurons (Cai et al. 2001). In mice, BACE1 cleaves APP at +1 and at +11 to yield $A\beta_{11-40/42}$ and $A\beta_{1-40-42}$. $A\beta$ beginning at the +11 site is the major species in rodent brains (Buxbaum et al. 1998).

PS1 and PS2 are ubiquitously expressed transmembrane proteins with six to nine transmembrane domains principally localized in the endoplasmic reticulum and Golgi (Selkoe 1998). Their function is unknown, although we know that deletions result in alterations in processing of APP such that $A\beta_{42}$ is increased (Scheuner et al. 1996). Although the role played by presenilins in the pathogenesis of sporadic AD remains unknown, mutations in the presenilin genes, especially PS-1, account for a significant proportion of cases of FAD.

Tau is one of the microtubule-associated proteins that stabilizes neuronal microtubules. The single gene that encodes tau generates six isoforms through alternate splicing. Each isoform contains either three (3R tau) or four (4R tau) consecutive imperfect repeat motifs of 31 or 32 amino acids in the carboxy terminal half of the protein. Tau is hydrophilic and soluble; however, when it is hyperphosphorylated on a number of serine and threonine residues, it is prone to aggregate into paired helical filaments that in turn form the neurofibrillary tangles and senile plaques typical of AD. After hyperphosphorylation, tau loses its ability to bind microtubules and is redistributed from the axon to a somatodendritic pattern (Goedert and Hasegawa 1999; Mandelkow and Mandelkow 1998).

In some cases of dementia, the clinical features of AD may overlap with those of other neurodegenerative diseases. Among the best characterized are cases that share clinical features of both AD and Parkinson's disease. In a significant number of such cases, the protein abnormalities and morphological changes characteristic of AD coexist with intraneuronal inclusions known as Lewy bodies, structures rich in β -synuclein, a protein normally expressed in synapses. Although a comprehensive review of the disorders associated with abnormal β -synuclein accumulation is beyond the scope of this article, recent observations suggest that the β -amyloid accumulations associated with AD may contribute to the development of β -synuclein-rich Lewy bodies in experimental animals. Whether β -synuclein, or other proteins, in turn influence the progression of AD-related changes remains unclear (Masliah et al. 2001).

β Amyloid Precursor Protein Transgenic Mice

Early efforts to create a transgenic mouse model of AD emphasized APP as the first protein found to have a genetic link to AD (Goate et al. 1991). Investigators hoped first to model AD and second to study the biological activity of APP overexpression in vivo. Factors that can affect the phenotype of transgenic mice expressing APP include the host strain, the primary structure of the APP, and the distribution and level of APP expression. Early paradigms used a number of neuron-specific promoters driving the expression of wild-type and AD APP in various murine backgrounds with limited success. The animals often died prematurely or failed to develop AD-like lesions in the brain (Hsiao et al. 1995; Quon et al. 1991). The first reported transgenic mouse was made using human APP under the control of neuron specific enolase. Although these animals had impaired memory and spatial alteration, they developed only rare $A\beta$ deposits in the brain (Moran et al. 1995). LaFerla used the FVB/N strain in which he expressed mouse $A\beta$, but the model was unsuccessful because >50% died within 1 yr. The mice developed corticolimbic gliosis, apoptosis, and extracellular $A\beta$ deposits (LaFerla et al. 1995).

Rather than emphasizing the extracellular fragment of APP, other investigators made transgenic mice that expressed the intracellular carboxy terminal 100 amino acid fragment of APP. In this model, the transgene is controlled by the brain dystrophin promoter, which directs expression to the hippocampus and neocortex (Neve et al. 1996; Oster-Granite et al. 1996). AP-C100 transgenic mice at 18 to 28 mo exhibited profound loss of neurons in Ammon's horn and the dentate gyrus, but none of the other classic features of Alzheimer's neuropathology developed (Oster-Granite et al. 1996). The finding that overexpression of the AP-C100 was neurotoxic is not surprising considering the recent findings that the C-terminal portion of APP is a component of a DNA transcription complex (Cao and Sudhof 2001) and its

overproduction could perturbate the expression of numerous genes.

PDAPP Mouse Model

Games and colleagues (1995) produced the first successful model known as the PDAPP mouse, which is now used extensively (Table 1). The PDAPP mouse expresses a human APP770 mini gene containing the V717F familial AD mutation (hAPP_{V717F}) under the control of the human platelet-derived growth factor (PDGF¹)-β chain neuronal pro-

motor on a mixed C57BL/6, DBA, and Swiss-Webster strain background. The success of this model was due to the construct used and the high level of APP expression achieved. The transgene contains a splicing cassette that permits expression of all three major APP isoforms. The PDGF-β promoter targets expression preferentially to neurons in the cortex, hippocampus, hypothalamus and cerebellum of the transgenic animals.

The familial AD mutation at residue 717 may be important because it partially shifts production of Aβ from the soluble 40-amino acid form to the more insoluble amyloidogenic 42-residue peptide known to predominate in AD

Table 1 Neuropathology in murine models of Alzheimer's disease

Mouse line (reference ^a)	Gene	Promoter	Comments
PDAPP ^b (Games et al. 1995)	Human APP770 with V717F mutation	PDGF-β	Amyloid deposits at 6-9 mo No neuronal loss No neurofibrillary tangles
Tg2576 (Hsiao et al. 1996)	Human APP695 isoform with double mutation K670N, M671L (hAPP _{Sw})	Hamster prion	Elevated Aβ 3 mo Amyloid deposits at 9-12 mo No neuronal loss No neurofibrillary tangles
APP23 (Sturchler-Pierrat et al. 1997)	Human APP695 isoform with double mutation K670N, M671L (hAPP _{Sw})	Murine Thy 1	Amyloid plaques at 6 mo Substantial neurodegeneration Hyperphosphorylated tau No neurofibrillary tangles
Tg2576 × BACE1 null (Luo et al. 2001)	As for Tg2576	As for Tg2576	Aβ -40 level in brain extracts drops to 5-7% of that for Tg2576
PS1 (Duff et al. 1996)	Human PS with mutation M146L or M146V	PDGF-β	Increased endogenous mouse Aβ 1-42/43 No neuropathology at 12 mo
APP × PS1 (Borchelt et al. 1997)	Human/mouse APP _{swe} with double mutation K595N, M596L Human PS1 with mutation A246E	Not reported	Elevated Aβ-142/Aβ 1-40 ratio in vitro Double transgenic mice develop amyloid deposits earlier than mice expressing either transgene alone.
Tg2576 × PS1 (Holcomb et al. 1998)	Human APP695 isoform with double mutation K670M, M671L (hAPP _{Sw}) Human PS1 with mutation M146L	Hamster prion	Increased Aβ 1-42/43 Amyloid deposits develop earlier than in single transgenic mice
Tau transgenic (Ishihara et al. 1999)	Human tau, shortest isoform (T44)	Mouse prion promoter	Argyrophilic inclusions by 12 mo of age Axonal degeneration Spinal cord gliosis and motor weakness Neuropathology more similar to FTDP-17 than AD
JNPL3 (Lewis et al. 2000)	Human four-repeat tau with mutation P301L	Mouse prion promoter	Tau-immunoreactive pre-tangles associated with neuronal degeneration in the spinal cord
Tg2576 × ApoE null (Kuo et al. 2000)	As for Tg2576	Hamster prion	Reduced Aβ burden compared with Tg2576
PDAPP × ApoE null (Irizarry et al. 2000)	As for PDAPP	PDGF-β	Character and distribution of amyloid deposits changed implicating ApoE in maturation of amyloid plaques

^aSee text.

^bApoE, apolipoprotein E; APP, amyloid precursor protein; PS, presenilin; PDAPP, amyloid precursor protein controlled by PDGF promoter; PDGF, platelet-derived growth factor.

plaques. A greater than 10-fold overexpression of human APP developed in this mouse. Cortical and limbic amyloid deposition began at 3 mo of age in homozygotes and at 6 to 9 mo in heterozygotes. The deposits were associated with reactive neuritic and inflammatory changes (Masliah et al. 1996). Although this model developed amyloid deposits, it failed to meet all criteria of the neuropathology of human AD in the absence of cortical or hippocampal neuronal loss or neurofibrillary tangles in aged transgenic animals (Irizarry et al. 1997b).

Tg2576 Mouse Model

Hsiao and colleagues (1996) produced the second popular transgenic model of AD known as the Tg2576 mouse. This mouse expresses human APP695 and contains the Swedish familial AD double mutation K670N, M671L (hAPP_{Sw}) controlled by the hamster prion protein (PrP) on a C57B6/SJL background. The mouse expresses human APP at a level more than sixfold higher than endogenous murine APP. The mice have a fivefold increase in the concentration of A β ₄₀ and a 14-fold increase in that of A β ₄₂. Both diffuse and dense core amyloid deposits developed in the same distribution as those found in the Games et al. (1995) mouse beginning in cortical and limbic regions by 9 mo of age. The amyloid deposits were associated with dystrophic neurites, punctate immunoreactivity to hyperphosphorylated tau, astrogliosis, microgliosis, and vascular amyloidosis, without significant CA1 neuronal loss or formation of neurofibrillary tangles (Irizarry et al. 1997a). The elevation of A β correlated with the appearance of memory and learning deficits in the oldest group of transgenic mice.

APP23 Mouse Model

The APP23 mouse (Bornemann and Staufenbiel 2000; Sturchler-Pierrat and Staufenbiel 2000; Sturchler-Pierrat et al. 1997) carries the same human Swedish double mutation at positions 670/671 as the Tg2576 mouse. Unlike the Tg2576 mouse, the APP23 mouse carries the APP751 isoform and the transgene is under the control of the murine Thyl promoter. These mice express human APP at a level sevenfold higher than murine endogenous APP. They developed amyloid plaques in the neocortex and hippocampus at 6 mo of age. The vast majority of the amyloid deposits were fibrillar. The plaques were almost exclusively congophilic at their first appearance and were associated with a pronounced glial reaction. A considerable amount of vascular amyloid was detected. Biochemical analysis revealed that the A β ₄₀ isoform was more prominent than the A β ₄₂ isoform. Both substantial neurodegeneration and a reduction of neuron numbers were apparent. Dystrophic neurites surrounded the plaques. Most importantly, hyperphosphorylated tau was detected in distorted neurites associated with

congophilic plaques. However, no neurofibrillary tangles developed.

General Considerations

Each of the models described above is remarkable in that the anatomical pattern of plaque formation parallels that seen in human AD. Furthermore, the morphology of amyloid plaques in aged APP transgenic mice recapitulates amyloid pathology in human AD inasmuch as the plaques span the continuum from diffuse A β deposits to compact core plaques with inflammation and neuritic dystrophy. However, none of these models reflects a complete picture of the neuropathology of AD because neurofibrillary tangles are not identified and prominent neurodegeneration and cerebral atrophy do not occur.

Further underscoring the difficulty of establishing a murine model of AD is a recent publication in which the authors report that the amyloid fibrils deposited in the brains of APP23 transgenic mice are chemically and morphologically distinct from those that develop in human brains with AD (Kuo et al. 2001). Mouse fibrils are completely soluble in buffers containing sodium dodecyl sulphate whereas human fibrils are insoluble. This difference occurs either because insufficient time is available in murine models for A β structural modifications to occur or because the complex species-specific environment of the human disease is not precisely replicated in the transgenic mouse. The authors caution that the evaluation of therapeutic agents or protocols must be considered in the context of the difference in plaques between the transgenic mouse and humans.

Recently the enzymes responsible for cleaving APP at the β -secretase site, BACE1 and BACE2, have been identified. Cai and colleagues (2001) established in neuronal cell cultures that BACE1, which is expressed at high levels in the brain, is the major β -secretase for the generation of A β peptides in neurons. In contrast, BACE2, which is expressed at very low levels in the brain, cleaves APP within the A β domain and precludes the formation of A β (Farzan et al. 2000). These *in vitro* studies suggest that BACE1 could be an exciting therapeutic target for protease inhibitors in AD. However, the following questions remain: (1) Would inhibition of BACE1 reduce the accumulation of A β peptides *in vivo*? (2) Would perturbation of BACE1 be neurotoxic? Knocking out BACE1 in mice proved that interfering with BACE1 did not have untoward effects. Mice deficient in BACE1 were healthy and fertile and appeared normal in gross anatomy, tissue histology, and clinical chemistry (Luo et al. 2001).

It is interesting that the BACE1 $-/-$ mouse is viable inasmuch as BACE1 is expressed in many tissues. The finding that there are no apparent adverse effects associated with BACE1 deficiency in mice suggests that inhibitors of BACE1 in humans may not be toxic. Although it was documented in neuronal cell cultures that BACE1-deficient cells produced less A β *in vitro*, transgenic animal experiments

would confirm whether or not similar changes would occur *in vivo*. When BACE1 $-/-$ mice were bred to Tg 2576 APP-overexpressing mice, which readily develop increased levels of A β in the brain by 3 mo of age (Hsiao et al. 1996), A β -40 levels in BACE1 $-/-$ APP+ brain extracts were only 5 to 7% of those in BACE1 $+/+$ APP+ mice.

Presenilin Transgenic Mice

Point mutations in the PS1 gene are a major cause of familial AD. Knocking out the gene offered no clues to its role in AD because mice null for PS1 die late in gestation (Shen et al. 1997). It has been proposed that the phenotype is a result of disturbed notch signaling. Because embryonic lethality precludes further analysis of the possible effects of PS1 on APP metabolism in living animals, brain cultures were generated from PS1 null embryos. *In vitro*, cleavage of the extracellular domain of APP by α - and β -secretase was not affected by the absence of PS1, but the activity of γ -secretase on the transmembrane domain of APP was prevented. This inhibition caused carboxyl-terminal fragments of APP to accumulate and resulted in a concurrent fivefold drop in the production of amyloid peptide (De Strooper et al. 1998).

PS1 Null Model

These *in vitro* findings support the idea that PS1 facilitates γ -secretase activity, which cleaves the integral membrane domain of APP, and that clinical mutations in PS1 result in a gain of the function of PS1. Further support for the gain of function theory is offered by knockin experiments using the PS1 null mouse (Qian et al. 1998). Transgenes expressing either the wild-type human PS1 or PS1 containing the FAD-associated mutation, A246E, under the transcriptional control of the human Thy-1 promoter, rescued the PS1 knockout mouse from embryonic lethality. Brain A β measurements revealed that mice expressing the mutant PS1 protein on the murine PS1 null background had a highly significant increase in the level of A β 1-42/43, whereas reduction of PS1 activity in heterozygous PS1 knockout mice did not lead to an increase in A β 1-42/43.

PDF Promoter Model

A second PS1 transgenic model expressing human mutant (M146L or M146V) and wild-type PS1 under the control of the PDGF promoter offered similar results (Duff et al. 1996). Expression of mutant or wild-type PS1 had no significant effect on brain A β 40, whereas expression of mutant but not wild-type PS1 increased endogenous mouse A β 1-42/43 in brain homogenates. Expression of wild-type PS1 did not significantly increase the levels of A β 1-42/43 even though expression of wild-type PS1 was substantially in-

creased to levels comparable with those for mutant PS1. Histopathological analysis of the mice at ages 3 to 4 wk revealed no A β deposition or other pathology. This finding is not surprising inasmuch as APP transgenic mice do not develop significant pathology until they are 12 mo old.

General Considerations

Because expression of mutant PS1 in mouse brain results in the accumulation of A β 42/43 from endogenous murine APP, the next logical step was to determine whether mutated PS1 would have a similar effect in mice transgenic for human APP. This question was studied by crossing mice expressing FAD-linked human PS1 variant (A246E) with a chimeric mouse/human APP harboring mutations (K595N, M596L) linked to Swedish FAD kindred (APP swe). The young double transgenic progeny had an elevated A β 1-42/A β 1-40 ratio in brain homogenates. The brains of transgenic mice expressing APP alone or transgenic mice coexpressing wild-type human PS1 and APP revealed no alteration.

These studies imply that mutant PS1 causes AD by increasing the extracellular concentration of A β 1-42/43 (Borchelt et al. 1996). Extension of these studies (Borchelt et al. 1997) to include neuropathological examination of the brain revealed that the mice transgenic for both genes developed numerous amyloid deposits much earlier than age-matched mice expressing APP swe and wild-type Hu PS1 and APP swe alone. Interestingly, the majority of A β deposits in the double transgenic mice were not immunoreactive with antisera against A β 1-42 but instead were stained with antisera to A β 1-40.

Crossing the Tg2576 transgenic mice, which express mutant APP_{K670N, M671L}, with mice transgenic for PS1_{M146L}, Holcomb and colleagues (1998) found that doubly transgenic mice revealed a selective 41% increase in A β 1-42/43 in homogenates of their brains. The AD-like pathology was substantially enhanced, and the mice revealed deterioration of brain function in the "y" maze.

Frequently double-transgenic mice have a complex genetic background in that the transgenic lines are on mixed backgrounds. To overcome this problem, Citron and colleagues (1997) bred a transgenic mouse bearing a human wild-type APP695 gene with mice bearing different human PS1 transgenes containing FAD-linked mutations to produce offspring expressing wild-type human APP695 alone or both wild-type APP95 and either mutant or wild-type human PS1. In this case, both genes were under the control of the cytomegalovirus promoter. Use of the same cytomegalovirus promoter element for both transgenes offered the advantage that both transgenes were likely to be expressed in adequate quantities in the same cells. In addition, both transgenic lines were on the same inbred FVB/N strain. With this strategy, the only difference between double and single transgenic animals was the presence or absence of the human PS1 transgene and any associated

insertional mutation. Mutant but not wild-type PS1 transgenic mice revealed significant overproduction of A β 42 in the brain, and this effect was detectable as early as 2 to 4 mo of age. These findings confirm that mutations in the presenilin gene cause a dominant gain of function and may induce AD by enhancing A β 42 production, thus promoting cerebral β -amyloidosis.

Tau Transgenic Mice

In addition to their presence in AD, filamentous tau inclusions accompanied by extensive gliosis and loss of neurons are the neuropathological hallmarks of an expanding family of other neurodegenerative diseases that are sometimes designated as tauopathies. The discovery of autosomal dominant pathogenic tau gene mutations in frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17¹) has led to the rapid emergence of new insights into mechanisms underlying FTDP-17, AD, and other tauopathies (Hutton et al. 1998).

Early efforts to produce animal models with tau pathology were based on the expectation that transgenic mouse models of AD that developed amyloid plaques would develop the other classic lesions of AD. However, tau-positive neurofibrillary tangles were never observed. Even the more complex transgenic mouse models (e.g., models that overexpressed FAD-linked PS1 mutations alone or FAD-linked PS1 and APP mutations together) did not develop tau pathology. Therefore, attention turned from classic mouse models for AD to mice transgenic for specific mutations of the tau protein.

Mutations in the tau gene in FTDP-17 can alter splicing and produce a shift from the short tau isoform with three repeats to the longer isoform, which contains four repeats. This finding suggests that overproduction of four-repeat tau may be sufficient to cause frontotemporal dementias. Reports from initial studies of mice transgenic for human tau indicated that the mice developed pretangle tau pathology but no filamentous tau inclusions (Brion et al. 1999; Gotz et al. 1995). The lack of tau filament formation in these transgenic models may have been due to production of a low level of human tau in that only 10 to 20% of total mouse brain tau was derived from the transgene.

Model of Shortest Human Tau Expression

Ishihara and colleagues (1999) were successful in creating a transgenic line that expressed high levels of the shortest human tau under the control of the mouse prion promoter. These mice developed insoluble intraneuronal filamentous hyperphosphorylated tau inclusions by 12 mo of age. These argyrophilic inclusions formed by aggregated 10- to 20-nm filaments composed of tau and neurofilament proteins were found in cortical and brainstem neurons but were most abundant in spinal cord neurons where they were associated with axon degeneration and reduced axonal transport in

ventral roots as well as spinal cord gliosis and motor weakness. However, the filaments within the inclusions did not exhibit the ultrastructural features of paired-helical filaments typical of classic AD neurofibrillary tangles. The phenotype of these transgenic mice was more similar to that seen in tauopathies such as FTDP-17 than to that seen in AD inasmuch as the tau pathologies were more abundant in the spinal cord and brain stem. In addition, these inclusions differed from authentic neurofibrillary tangles (NFTs¹) in AD and other human tauopathies in that the tau inclusions were not stained by thioflavin-S or Congo red and contained straight 10- to 20-nm-diameter filaments that were admixed with neurofilaments. Even though tau protein isolated from brain and spinal cord of these mice became progressively insoluble and more phosphorylated with age, overexpression of human tau did not result in neurofibrillary tangles. If the ages of the mice described above were beyond 12 mo, however, the character and distribution of the inclusions changed (Ishihara et al. 2001). In contrast to those in younger animals, the inclusions were congophilic, similar to those found in human tauopathies. Ultrastructurally, the lesions contained straight tau filaments composed of both mouse and human tau proteins but not other cytoskeletal proteins. The tau inclusions developed in the hippocampus and associated limbic areas.

JNPL3 Mouse Model

Transgenic mice designated JNPL3 express human four-repeat tau containing the most common FTDP-17 mutation (P301L) under the control of the mouse prion promoter and developed motor and behavioral deficits (Lewis et al. 2000). These deficits were associated with age- and gene-dose-dependent development of congophilic neurofibrillary tangles, which formed in the amygdala, septal nuclei, preoptic nuclei, hypothalamus, midbrain, pons, medulla, deep cerebellar nuclei, and spinal cord. Tau-immunoreactive pretangles were found in the cortex, hippocampus, and basal ganglia, but at a lower level than commonly found in human disease. The tangles were associated with neuronal degeneration, especially in the spinal cord where motor neurons were reduced by approximately 48%. Interestingly, this transgenic model had a surprisingly severe phenotype given the relatively low-level expression of the transgene. This severity presumably reflects the inclusion of the P301L mutation that is associated with FTDP-17. Phenotypically, this mouse recapitulates features of the human tauopathies rather than the classic features of AD. However, it will be valuable in breeding experiments to produce model systems that more accurately recapitulate the hallmark pathology of AD by crossing it with transgenic mouse models of AD A β amyloidosis.

Apolipoprotein E Transgenic Mice

Apolipoprotein E (ApoE¹) appears to play an important role in the pathogenesis of AD inasmuch as the relative risk of

developing late-onset senile dementia of the AD type is increased in individuals who inherit an *APOEε4* allele. A consistent consequence of carrying the *APOEε4* allele is an increased number of amyloid plaques in brain and more abundant amyloid deposition in the cerebral vasculature. The mechanism by which ApoE4 contributes to the development of neurodegeneration remains unknown, but it may modify the ability of the brain to respond to environmental stresses or alter the blood-brain barrier (Strittmatter 2000).

Employing a knockout strategy, two distinct AD models, Tg2576 and PDAPP, have been crossed with ApoE null mice to determine whether the lack of ApoE would affect the neuropathological phenotype. The ApoE null animals revealed that the absence of ApoE altered the quantity, character, and distribution of Aβ deposits in the transgenic animals.

Tg2576xApoe Mouse Model

As Aβ accumulates in the brains of Tg2576 transgenic mice, the brain ApoE increases by 60% relative to control mice. The ApoE accumulates in neuritic plaques that are thioflavin-S positive, suggesting that elevation of brain ApoE in Tg2576 mice participates in an age-related abnormal regulation of Aβ clearance (Kuo et al. 2000).

When examined at 1 yr of age, mice heterozygous for APP on an ApoE null background had significantly reduced Aβ deposition, lacked thioflavine-S-positive deposits, had no neurodegeneration, and developed less vascular amyloid compared with heterozygous mice with endogenous mouse ApoE. The pattern of amyloid deposits in the cortex and hippocampus did not change in the ApoE null background.

PDAPPxApoe Mouse Model

In experiments crossing PDAPP homozygous mice (Games et al. 1995) with ApoE null mice, the Aβ burden in the cortex and hippocampus was markedly reduced. Interestingly, the character of the plaques changed in this model. Elimination of ApoE prevented the formation of compact, thioflavine-S-staining plaques.

In animals examined at 12 mo of age, a dramatic redistribution of deposits occurred. PDAPP mice with ApoE had compact deposits scattered throughout the frontal cortex. PDAPP^{+/+}ApoE^{-/-} mice had diffuse deposits only in the deep cortical layers. Within the hippocampal subfields, the pattern of Aβ staining in the ApoE null mice was altered in a very specific anatomic manner. In PDAPP^{+/+}ApoE^{+/+} mice, amyloid deposited prominently in a band in the outer layer of the dentate gyrus, with focal deposits throughout the other hippocampal subfields as in human AD. In ApoE null mice, the dentate gyrus was remarkably free of Aβ immunoreactivity.

The findings that the levels of APP mRNA by RT-polymerase chain reaction, the levels of APP protein by

Western blot, or total Aβ and Aβ1-42 by enzyme-linked immunosorbent assay in the hippocampus or cortex did not change in the ApoE null background implicate ApoE in Aβ fibrillogenesis, stabilization of fibrillar Aβ, and/or maturation of amyloid plaques (Bales et al. 1997; Irizarry et al. 2000). In contrast, transgenic experiments wherein ApoE was overexpressed yield entirely different results from those described in ApoE knockout mice. Overexpression of the human *APOE4* allele in neurons resulted in hyperphosphorylation of protein tau (Tesseur et al. 2000b). In three independent transgenic lines using two different promoter constructs, increased phosphorylation of protein tau was correlated with ApoE expression levels. Hyperphosphorylation of tau increased with age. These findings suggest a role for ApoE4 in neuronal cytoskeletal stability and metabolism. No neurofibrillary tangles or other neurofibrillary inclusions were found; however, axonal dilations with accumulation of synaptophysin, neurofilaments, mitochondria, and vesicles were documented, suggesting impairment of axonal transport (Tesseur et al. 2000a). This current transgenic mouse offers the opportunity to investigate the interaction between ApoE 4 and protein tau.

Nepriylsin Transgenic Mice

At the time of this writing, most research using transgenic mouse models of AD has focused on the secretases that free Aβ from APP. However, investigators have recently discovered two proteases that actively degrade Aβ: insulin-degrading protein (Vekrellis et al. 2000) and neprilysin (Shirovani et al. 2001; Takaki et al. 2000). If these enzymes were defective, Aβ could theoretically accumulate.

Nepriylsin-null mice offer direct evidence that neprilysin could be a natural Aβ-degrading enzyme (Iwata et al. 2001). When Aβ is given by injection into the brains of normal mice, it is degraded in about 30 min; however, in neprilysin knockout mice, almost all of the peptide persists. In mice heterozygous for neprilysin, more of the injected Aβ persisted than in the wild-type, but less than in the null. The Aβ levels in knockout mice were highest in the hippocampus and cortex, where Alzheimer's plaques are most prominent. Relevance of neprilysin to AD in humans was suggested when it was discovered that there are low levels of neprilysin in plaque regions in patients who died of AD (Yasojima et al. 2001). With the availability of transgenic mice that accumulate Aβ in the brain, it will be of interest to determine whether the accumulation of Aβ can be accelerated by breeding them to neprilysin null mice.

Aβ Immunization and AD

Clearly, transgenic mice have played a pivotal role in understanding the various molecular elements in the pathogenesis of AD. A significant recent development has been the use of these models to evaluate treatment modalities. Given

the paucity of inflammatory changes in AD, it is both surprising and exciting to find that vaccination with A β ₄₂ can alter the course of plaque formation in murine models of AD. Immunization with A β ₄₂ offered promising results either at 6 wk of age, before the onset of AD-type neuropathology, or at 11 mo, during amyloid- β deposition (Schenk et al. 1999). The immunization of young animals essentially prevented the development of β -amyloid plaque formation, neuritic dystrophy, and astrogliosis. Treatment of older animals also markedly reduced the extent and progression of the AD-like neuropathology.

There are two possible mechanisms to account for the success of the vaccination protocol. The anti-A β antibodies could reduce the plaques either by facilitating clearance of amyloid- β before deposition or by triggering monocytic/microglial cells to clear established plaques through signals mediated by Fc receptors. The latter mechanism dominates. Studies using the same model revealed that antibodies to A β cross the blood-brain barrier and decorate the plaques triggering microglial cells to clear plaques through Fc receptor-mediated phagocytosis and subsequent peptide degradation (Bard et al. 2000).

Vaccination has an obvious effect not only on the morphology of β -amyloid plaques but also on the amelioration of cognitive dysfunction. Using Tg 2576 APP transgenic mice (Hsiao et al. 1996) crossed to PS1 transgenic mice (Duff et al. 1996) that have age-related cognitive impairment (Arendash et al. 2001), investigators found that vaccinated mice performed in cognitive testing as well as nontransgenic mice (Morgan et al. 2000). Parallel cognitive improvement was also seen in the TgCRND8 murine model. This mouse is a modified Tg2576 mouse that carries a double mutated human APP- β (K670N, M671L and V717F) on a C3H/B6 background (Janus et al. 2000).

Conclusions

It is clear that none of the mouse models to date recapitulate the complete neuropathology of AD. Some models develop plaques and others, NFTs and neurodegeneration. Nonetheless, the genetically altered mouse has offered tremendous insight into the function of the various molecular elements in the pathogenesis of AD, although questions remain. It is still unclear as to how A β and tau are related. Two camps exist, each purporting the primacy of either tau or A β . The existing models offer a solid platform from which to explore this debate, and two recently published papers help bring the two camps together.

Gotz and colleagues (2001) describe a model wherein P301L tau transgenic mice develop neurofibrillary tangles after the intracerebral injection of A β ₄₂. The A β was given by injection into the CA1 region of the hippocampus, and the neurofibrillary tangles developed in the respective cell bodies of projection neurons in the amygdala as soon as 18 days after A β injection. These experiments indicate that the interaction of β -amyloid with the P301L mutation is re-

quired for NFT formation. Neither β -amyloid nor the mutation in tau alone is sufficient to generate high numbers of NFTs. It will be interesting to learn whether vaccination with A β will be effective in preventing NFT formation in this model.

Double mutants produced from crossing JNPL3 transgenic mice expressing mutant P301L tau (Lewis et al. 2000) with Tg 2576 mice (Hsiao et al. 1996) expressing the APP Swedish mutation also offered proof that A β influences the development of NFTs (Lewis et al. 2001). The double mutants exhibited neurofibrillary tangle pathology that was substantially enhanced in the limbic system and the olfactory cortex. These results suggest that APP or A β augments the formation of neurofibrillary tangles in the regions of the brain vulnerable to the formation of these lesions. This model most closely recapitulates the lesions of AD in humans inasmuch as the mice develop not only amyloid deposition and NFTs but also neuronal loss. This model is not likely to be the final murine model of AD. It and other models to come will be invaluable in determining the pathogenesis of AD and in evaluating therapeutic protocols designed to prevent the disease.

References

- Arendash GW, King DL, Gordon MN, Morgan D, Hatcher JM, Hope GE, Diamond DM. 2001. Progressive, age-related behavioral impairments in transgenic mice carrying both mutant amyloid precursor protein and presenilin-1 transgenes. *Brain Res* 891:42-53.
- Bales KR, Verina T, Dodel RC, Du Y, Altstiel L, Bender M, Hyslop P, Johnstone EM, Little SP, Cummins DJ, Piccardo P, Ghetti B, Paul SM. 1997. Lack of apolipoprotein E dramatically reduces amyloid beta-peptide deposition. *Nat Genet* 17:263-264.
- Bard F, Cannon C, Barbour R, Burke RL, Games D, Grajeda H, Guido T, Hu K, Huang I, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Lieberburg I, Motter R, Nguyen M, Soriano F, Vasquez N, Weiss K, Welch B, Seubert, Schenk D, Yednock T. 2000. Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer's disease. *Nat Med* 6:916-919.
- Borchelt DR, Ratovitski, T, van Lare J, Lee MK, Gonzales V, Jenkins NA, Copeland NG, Price DL, Sisodia SS. 1997. Accelerated amyloid deposition in the brains of transgenic mice coexpressing mutant presenilin 1 and amyloid precursor proteins. *Neuron* 19:939-945.
- Borchelt DR, Thinakaran G, Eckman CB, Lee MK, Davenport F, Ratovitsky T, Prada CM, Kim G, Seekins S, Yager D, Slunt HH, Wang R, Seeger M, Levey AI, Gandy SE, Copeland NG, Jenkins NA, Price DL, Younkin SG, Sisodia SS. 1996. Familial Alzheimer's disease-linked presenilin I variants elevate Abeta1-42/1-40 ratio in vitro and in vivo. *Neuron* 17:1005-1013.
- Bornemann KD, Staufenbiel M. 2000. Transgenic mouse models of Alzheimer's disease. *Ann NY Acad Sci* 908:260-266.
- Brion JP, Tremp G, Octave JN. 1999. Transgenic expression of the shortest human tau affects its compartmentalization and its phosphorylation as in the pretangle stage of Alzheimer's disease. *Am J Pathol* 154:255-270.
- Buxbaum JD, Thinakaran G, Koliatsos V, O'Callahan J, Slunt HH, Price DL, Sisodia SS. 1998. Alzheimer amyloid protein precursor in the rat hippocampus: Transport and processing through the perforant path. *J Neurosci* 18:9629-9637.
- Cai H, Wang Y, McCarthy D, Wen H, Borchelt DR, Price DL, Wong PC.

2001. BACE1 is the major β -secretase for generation of A β peptides by neurons. *Nat Neurosci* 4:233-234.
- Cao X, Sudhof TC. 2001. A transcriptionally active complex of app with fe65 and histone acetyltransferase tip60. *Science* 293:115-120.
- Citron M, Westaway D, Xia W, Carlson G, Diehi T, Levesque G, Johnson-Wood K, Lee M, Seubert P, Davis A, Kholodenko D, Motter R, Sherrington R, Perry B, Yao H, Strome R, Lieberburg I, Rommens J, Kim S, Schenk D, Fraser P, St George-Hyslop P, Selkoe DJ. 1997. Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid beta-protein in both transfected cells and transgenic mice. *Nat Med* 3:67-72.
- De Strooper B, Saftig P, Craessaerts K, Vanderstichele H, Guhde G, Annaert W, Von Figura K, Van Leuven F. 1998. Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature* 391:387-390.
- Duff K, Eckman C, Zehr C, Yu X, Prada CM, Perez-tur J, Hutton M, Buee L, Harigaya Y, Yager D, Morgan D, Gordon MN, Holcomb L, Refolo L, Zenk B, Hardy J, Younkin S. 1996. Increased amyloid-beta(42/43) in brains of mice expressing mutant presenilin 1. *Nature* 383:710-713.
- Farzan M, Schnitzler CE, Vasilieva N, Leung D, Choe H. 2000. BACE2, a β -secretase homolog, cleaves at the β site and within the amyloid- β region of the amyloid- β precursor protein. *Proc Natl Acad Sci U S A* 97:9712-9717.
- Games D, Adams D, Alessandrini R, Barbour R, Berthelette P, Blackwell C, Carr T, Clemens J, Donaldson T, Gillespie F, Guido T, Hagoplan S, Johnson-Wood K, Khan K, Lee M, Leibowitz P, Lieberburg I, Little S, Masliah E, McConlogue L, Montoya-Zavala M, Mucke L, Paganini L, Penniman E, Power M, Shenk D, Seubert P, Snyder B, Soriano F, Tan H, Vitale J, Wadsworth S, Wolozin B, Zhao J. 1995. Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. *Nature* 373:523-527.
- Goate A, Chartier-Harlin M-C, Mullan M, Brown J, Crawford F, Fidani L, Giuffra L, Haynes A, Irving N, James L, Mant R, Newton P, Rooke K, Roques P, Talbot C, Pericak-Vance M, Roses A, Williamson R, Rossor M, Owen M, Hardy J. 1991. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature* 349:704-713.
- Goedert M, Hasegawa M. 1999. The tauopathies: Toward an experimental animal model. *Am J Pathol* 154:1-6.
- Gotz J, Chen F, van Dorpe J, Nitsch RM. 2001. Formation of neurofibrillary tangles of P301L tau transgenic mice induced by A β 42 fibrils. *Science* 293:1491-1495.
- Gotz J, Probst A, Spillantini MG, Schafer T, Jakes R, Burki K, Goedert M. 1995. Somatodendritic localization and hyperphosphorylation of tau protein in transgenic mice expressing the longest human brain tau isoform. *Embo J* 14:1304-1313.
- Holcomb L, Gordon MN, McGowan E, Yu X, Benkovic S, Jantzen P, Wright K, Saad I, Mueller R, Morgan D, Sanders S, Zehr C, O'Campo K, Hardy J, Prada CM, Eckman C, Younkin S, Hsiao K, Duff K. 1998. Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nat Med* 4:97-100.
- Hsiao KK, Borchelt DR, Olson K, Johanrisdottir R, Kitt C, Yunis W, Xu S, Eckman C, Younkin S, Price D, Iadecola C, Clark HB, Carlson G. 1995. Age-related CNS disorder and early death in transgenic FVB/N mice overexpressing Alzheimer amyloid precursor proteins. *Neuron* 15:1203-1218.
- Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F, Cole G. 1996. Correlative memory deficits, A beta elevation, and amyloid plaques in transgenic mice. *Science* 274:99-102.
- Hutton M, Lendon L, Rizzu P, Baker M, Froelich S, Houlden H, Pickering-Brown S, Chakraverty S, Isaacs A, Grover A, Hackett J, Adamson J, Lincoln S, Dickson D, Davies P, Petersen RC, Stevens M, de Graaff E, Wauters E, van Varen J, Hillebrand M, Joosse M, Kwon JM, Nowotny P, Che LK, Norton J, Morris JC, Reed LA, Trojanowski J, Basun H, Lannfelt L, Naystat M, Fahn S, Dark F, Tannenberg T, Dodd PR, Hayward N, Kwok JBJ, Schofield PR, Andreadis A, Snowden J, Craufurd D, Neary D, Owen F, Oostra BA, Hardy J, Goate A, van Swieten J, Mann D, Lynch T, Heutink P. 1998. Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature* 393:702-705.
- Hyman BT, Trojanowski JQ. 1997. Editorial on consensus recommendations for the postmortem diagnosis of Alzheimer disease from the National Institute on Aging and the Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer Disease. *J Neuropathol Exp Neurol* 56:1095-1097.
- Irizarry MC, Cheurig BS, Rebeck GW, Paul SM, Bales KR, Hyman BT. 2000. Apolipoprotein E affects the amount, form, and anatomical distribution of amyloid beta-peptide deposition in homozygous APP(V717F) transgenic mice. *Acta Neuropathol (Berl)* 100:451-458.
- Irizarry MC, McNamara M, Fedorchak K, Hsiao K, Hyman BT. 1997a. APPSw transgenic mice develop age-related A beta deposits and neuropil abnormalities, but no neuronal loss in CA1. *J Neuropathol Exp Neurol* 56:965-973.
- Irizarry MC, Soriano F, McNamara M, Page KJ, Schenk D, Games D, Hyman BT. 1997b. A-beta deposition is associated with neuropil changes, but not with overt neuronal loss in the human amyloid precursor protein V717F (PDAPP) transgenic mouse. *J Neurosci* 17:7053-7059.
- Ishihara T, Hong M, Zhang B, Nakagawa Y, Lee MK, Trojanowski Q, Lee VM. 1999. Age-dependent emergence and progression of a tauopathy in transgenic mice overexpressing the shortest human tau isoform. *Neuron* 24:751-762.
- Ishihara T, Zhang B, Higuchi M, Yoshiyama Y, Trojanowski JQ, Lee VM. 2001. Age-dependent induction of congophilic neurofibrillary tau inclusions in tau transgenic mice. *Am J Pathol* 158:555-562.
- Iwata N, Tsubuki S, Takaki Y, Shirota K, Lu B, Gerard NP, Gerard C, Hama E, Lee HJ, Saido TC. 2001. Metabolic regulation of brain A-beta by neprilysin. *Science* 292:1550-1552.
- Janus C, Pearson J, McLaurin J, Mathews PM, Jiang S Y, Schmidt D, Chishti MA, Horne P, Heslin D, French J, Mount HT, Nixon RA, Mercken M, Bergeron C, Fraser PE, St George-Hyslop P, Westaway D. 2000. A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. *Nature* 408:979-982.
- Kuo YM, Crawford F, Mullan M, Kokjohn TA, Emmerling MR, Weller RO, Roher AE. 2000. Elevated A beta and apolipoprotein E in A betaPP transgenic mice and its relationship to amyloid accumulation in Alzheimer's disease. *Mol Med* 6:430-439.
- Kuo YM, Kokjohn TA, Beach TG, Sue LI, Brune D, Lopez JC, Kalback WM, Abramowski D, Sturchler-Pierrat C, Staufenbiel M, Roher AE. 2001. Comparative analysis of amyloid- β chemical structure and amyloid plaque morphology of transgenic mouse and Alzheimer's disease brains. *J Biol Chem* 276:12991-12998.
- LaFerla FM, Tinkle BT, Bieberich CJ, Haudenschield CC, Jay G. 1995. The Alzheimer's A beta peptide induces neurodegeneration and apoptotic cell death in transgenic mice. *Nat Genet* 9:21-30.
- Lewis J, Dickson DW, Lin W-L, Chisholm L, Corral A, Jones G, Yen S-H, Sahara N, Skipper L, Yager D, Echman C, Hardy J, Hutton M, McGowan E. 2001. Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. *Science* 293:1487-1491.
- Lewis J, McGowan E, Rockwood J, Melrose H, Nachamaju P, Van Slegtenhorst M, Gwinn-Hardy K, Murphy MP, Baker M, Yu X, Duff K, Hardy J, Corral A, Lin WL, Yen SH, Dickson DW, Davies P, Hutton M, Nasir J, Floresco SB, O'Kusky JR, Diewert VM, Richman JM, Zeisler J, Borowski A, Marth JD, Phillips AG, Hayden MR. 2000. Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein. *Nat Genet* 25:402-405.
- Luo Y, Bolon B, Kahn S, Bennett BD, Babu-Khan S, Denis P, Fan W, Kha H, Zhang J, Gong Y, Martin L, Louis JC, Yan Q, Richards WG, Citron M, Vassar R. 2001. Mice deficient in BACE1, the Alzheimer's beta-secretase, have normal phenotype and abolished beta-amyloid generation. *Nat Neurosci* 4:231-232.
- Mandelkow EM, Mandelkow F. 1998. Tau in Alzheimer's disease. *Trends Cell Biol* 8:425-427.
- Masliah E, Rockenstein E, Veinbergs I, Yutaka S, Mallory M, Hashimoto M, Mucke L. 2001. β -Amyloid peptides enhance α -synuclein accumu-

- lation and neuronal deficits in a transgenic mouse model linking Alzheimer's disease and Parkinson's disease. *Proc Natl Acad Sci U S A* 98: 12245-12250.
- Masliah E, Sisk A, Mallory M, Mucke L, Schenk D, Games D. 1996. Comparison of neurodegenerative pathology in transgenic mice overexpressing V717F beta-amyloid precursor protein and Alzheimer's disease. *J Neurosci* 16:5795-5811.
- Moran PM, Higgins LS, Cordell B, Moser PC. 1995. Age-related learning deficits in transgenic mice expressing the 751-amino acid isoform of human beta-amyloid precursor protein. *Proc Natl Acad Sci U S A* 92:5341-5345.
- Morgan D, Diamond DM, Gottschall PE, Ugen KE, Dickey C, Hardy J, Duff K, Jantzen P, DiCarlo G, Wilcock D, Connor K, Hatcher J, Hope C, Gordon M, Arendash GW. 2000. A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature* 408:982-985.
- Neve RL, Boyce FM, McPhie DL, Greenan J, Oster-Granite ML. 1996. Transgenic mice expressing APP-C100 in the brain. *Neurobiol Aging* 17:191-203.
- Oster-Granite ML, McPhie DL, Greenan J, Neve RL. 1996. Age-dependent neuronal and synaptic degeneration in mice transgenic for the C terminus of the amyloid precursor protein. *J Neurosci* 16:6732-6741.
- Qian S, Jiang P, Guan XM, Singh G, Trumbauer ME, Yu H, Chen HY, Van de Ploeg LH, Zheng H. 1998. Mutant human presenilin I protects presenilin I null mouse against embryonic lethality and elevates A β 1-42/43 expression. *Neuron* 20:611-617.
- Quon D, Wang Y, Catalano R, Scardina JM, Murakami K, Cordell B. 1991. Formation of beta-amyloid protein deposits in brains of transgenic mice. *Nature* 352:239-241.
- Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, Hu K, Huang, J, Johnson-Wood K, Khan K, Kholodenko D, Lee Z, Liao M, Lieberburg I, Motter R, Mutter L, Soriano F, Shopp G, Vasquez N, Vandeventer C, Walker S, Wogulis M, Yednock T, Games D, Seubert P. 1999. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* 400:173-177.
- Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N, Bird TD, Hardy J, Hutton M, Kukull W, Larson E, Levy-Lahad E, Viitanen M, Peskind E, Poorkaj P, Schellenberg G, Tanzi R, Wasco W, Lannfelt L, Selkoe D, Younkin S. 1996. Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat Med* 2:864-870.
- Selkoe DJ. 1998. The cell biology of beta-amyloid precursor protein and presenilin in Alzheimer's disease. *Trends Cell Biol* 8:447-453.
- Shen J, Bronson RT, Chen DF, Xia W, Selkoe DJ, Tonegawa S. 1997. Skeletal and CNS defects in presenilin-1-deficient mice. *Cell* 89:629-639.
- Shirovani K, Tsubuki S, Iwata N, Takaki Y, Harigaya W, Maruyama K, Kiryu-Seo, S, Kiyama H, Iwata H, Tomita T, Iwatsubo T, Saido TC. 2001. Nephilysin degrades both amyloid- β peptides 1-40 and 1-42 most rapidly and efficiently among thiorphan- and phosphoramidon-sensitive endopeptidases. *J Biol Chem* 276:21895-21901.
- Sinha S, Anderson JP, Barbour R, Basu GS, Caccavello R, Davis D, Doan M, Dovey HF, Frigon N, Hong J, Jacobson-Croak K, Jewett N, Keim P, Knops J, Lieberburg I, Power M, Tan H, Tatsuno G, Tung J, Schenk D, Seubert P, Suomensaaari SM, Wang S, Walker D, Zhao J, McConlogue L, Varghese J. 1999. Purification and cloning of amyloid precursor protein beta-secretase from human brain. *Nature* 402:537-540.
- Strittmatter WJ. 2000. Apolipoprotein E and Alzheimer's disease. *Ann N Y Acad Sci* 924:91-92.
- Sturchler-Pierrat C, Abramowski D, Duke M, Wiederhold KH, Mistl C, Rothacher S, Ledermann B, Burki K, Frey P, Paganetti PA, Waridel C, Calhoun ME, Jucker M, Probst A, Staufenbiel M, Sommer B, Diewert VM, Richman JM, Zeisler J, Borowski A, Marth JD, Phillips AG, Hayden MR. 1997. Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. *Proc Natl Acad Sci U S A* 94:13287-13292.
- Sturchler-Pierrat C, Staufenbiel M. 2000. Pathogenic mechanisms of Alzheimer's disease analyzed in the APP23 transgenic mouse model. *Ann N Y Acad Sci* 920:134-139.
- Takaki Y, Iwata N, Tsubuki S, Taniguchi S, Toyoshima S, Lu B, Gerard NP, Gerard C, Lee HJ, Shirovani K, Saido TC. 2000. Biochemical identification of the neutral endopeptidase family member responsible for the catabolism of amyloid beta peptide in the brain. *J Biochem* 128:897-902.
- Tesseur I, Van Dorpe J, Bruynseels K, Bronfman F, Sciote R, Van Lommel A, Van Leuven F. 2000a. Prominent axonopathy and disruption of axonal transport in transgenic mice expressing human apolipoprotein E4 in neurons of brain and spinal cord. *Am J Pathol* 157:1495-1510.
- Tesseur I, Van Dorpe J, Spittaels K, Van den Haute C, Moechars D, Van Leuven F. 2000b. Expression of human apolipoprotein E4 in neurons causes hyperphosphorylation of protein tau in the brains of transgenic mice. *Am J Pathol* 156:951-964.
- Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, Teplow DB, Ross S, Amarante P, Loeloff R, Luo Y, Fisher S, Fuller J, Edenson S, Lile J, Jarosinski MA, Biere AL, Curran E, Burgess T, Louis JC, Collins F, Treanor J, Rogers G, Citron M. 1999. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* 286:735-741.
- Vekrellis K, Ye Z, Qiu WQ, Walsh D, Hartley D, Chesneau V, Rosner MR, Selkoe DJ. 2000. Neurons regulate extracellular levels of amyloid beta-protein via proteolysis by insulin-degrading enzyme. *J Neurosci* 20: 1657-1665.
- Yan R, Bienkowski MJ, Shuck ME, Miao H, Tory MC, Pauley AM, Brashier JR, Stratman NC, Mathews WR, Buhl AE, Carter DB, Tomasselli AG, Parodi LA, Heinrichson RL, Gurney ME. 1999. Membrane-anchored aspartyl protease with Alzheimer's disease beta-secretase activity. *Nature* 402:533-537.
- Yasojima K, Akiyama H, McGeer EG, McGeer PL. 2001. Reduced neprilysin in high plaque areas of Alzheimer brain: A possible relationship to deficient degradation of beta-amyloid peptide. *Neurosci Lett* 297: 97-100.
- Zheng H, Jiang M, Trumbauer ME, Sirinathsinghji DJ, Hopkins R, Smith DW, Heavens RP, Dawson GR, Boyce S, Conner MW, Stevens KA, Slunt HH, Sisodia SS, Chen HY, Van der Ploeg LH. 1995. Beta-amyloid precursor protein-deficient mice reveal reactive gliosis and decreased locomotor activity. *Cell* 81:525-531.