

Transgenic Mice Model in Alzheimer's Disease

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Abstract

Alzheimer's disease can either occur sporadically or be caused by inheritance of a gene mutation accompanying progressive loss of memory and general decline in cognition. The pathology of Alzheimer's disease is complex, but there are 2 hallmarks: the neuritic plaque consisting of β -amyloid derived from β -amyloid precursor proteins and the neurofibrillary tangles consisting of abnormally phosphorylated tau protein. Transgenic mice are used to reproduce these 2 hallmarks since they can reproduce many features of the disease. This review provides an overview of transgenic mouse models including familial Alzheimer's disease and non-hereditary Alzheimer's disease as well as emerging insights relevant to the pathogenesis and potential treatment strategies.

Key words: Alzheimer disease, Animal models, Therapy, Transgenic mice

Introduction

Alzheimer's disease (AD) is the most prevalent type of dementia. It occurs either sporadically or is caused by inheritance of a gene mutation accompanying progressive loss of memory and global cognitive decline. The pathology of AD is complex, but there are 2 hallmarks that occur abundantly in most cases. The neuritic plaque is a largely extracellular lesion consisting of β -amyloid ($A\beta$) released from cleaved $A\beta$ precursor proteins (APPs) by α -, β - and γ -secretase and the intracellular neurofibrillary tangles (NFTs) consist largely of abnormally phosphorylated tau protein. Neuronal and synaptic loss with reactive gliosis also occur.

A good animal model will help considerably both for understanding the relationships between various aspects of pathology and for testing therapies based upon these relationships.¹

Hereditary Alzheimer's Disease Transgenic Animal Model

Alzheimer's Disease Genetics

Some cases of early onset AD are familial autosomal dominant (FAD) disorders caused by mutations in APP, PS1

and PS2.² In the late onset forms, there are no specific gene mutations associated with FAD inheritance, although specific alleles of apolipoprotein E and $\alpha 2$ macroglobulin (A2M) are reported to be associated with increased risk for AD (Table 1).³

Transgenic mice that express disease-causing genes reproduce many features of the disease. These models are proving to be useful in investigations of the nature of biochemical alterations in neural tissue, the character and evolution of pathologies, and the pathogenic mechanisms.

β -Amyloid Precursor Protein Mutations

The APP gene, the first AD susceptibility gene to be identified, encodes a transmembrane protein that contains 770 amino acids in its longest isoform. Mutational studies of the APP gene have identified a Glu693Gln missense mutation of the APP gene,⁴ and several other missense mutations in exons 16 and 17 of the APP gene. Although a number of APP mutations have been identified, most are probably not disease causing. However, the missense mutations at codon 670/671 (Swedish mutation), at codon 692 (Flemish mutation), at codon 716, and at codon 717 are thought to be pathogenic.

The first successful transgenic mouse model with $A\beta$ amyloid deposition was made by Games et al.⁵ These researchers took an unusual construct that included full-length human APP complementary DNA with the APP717Val(V) \rightarrow Phe(F) mutation under the control of the platelet-derived growth factor promoter, which targets expression preferentially to neurones of the cortex, hippocampus, hypothalamus, and cerebellum of transgenic animals.⁶ The pathology of the mice was as striking as in patients with AD, and included extracellular $A\beta$ deposition, dystrophic component, gliosis, and loss of synaptic density with regional specificity resembling that of AD, although NFTs were not evident.

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Table 1. Relationship between genotype, histopathology and Alzheimer's disease (AD).

AD	Linkage	Gene	Mutation	Pathology
Early onset	Ch21	App	Missense in and around A β region of APP, increase A β or A β 42	Amyloid plaques
	Ch14	PS1	Mainly missense, increase A β 42	Amyloid plaques and T
	Ch1	PS2	Missense, increase A β 42	Amyloid plaques and T
Late onset	Ch12	A2M		
	Ch19	ApoE4		

Abbreviations: Ch = chromosome; APP = β -amyloid precursor protein; PS1 = actual disease gene; PS2 = homologue; A2M = α 2 macroglobulin; ApoE = apolipoprotein E; A β = β -amyloid; T = tangles.

Hsiao et al used the hamster prion protein promoter to overexpress the human APP₆₉₅ containing Lys670→Asn, Met671→Len mutation (Swedish mutation).⁷ These transgenic mice had normal learning and memory in spatial reference and alternation tasks at the age of 3 months, but showed impairment by the age of 9 to 10 months. This impairment was correlated with a marked increase in the amounts of A β and was accompanied by numerous amyloid plaques and A β deposits.

The transgenic mice with robust behavioural and pathological features resembling those found in AD offer an appropriate tool for exploring the pathophysiology and neurobiology of this disease.⁷ Both studies support the amyloid cascade hypothesis of AD pathogenesis, which states that production and deposition of A β in the form of fibrils leads to neuronal cell death and eventually to clinical presentation and progression of AD.⁸

Genetic strategies have also been used to gain insight into mechanisms of the disease. A controversial question still exists as to whether A β -induced neurotoxicity requires deposition of aggregated A β into plaques.⁹⁻¹² Hsiao et al postulated that the neurotoxic effect of A β is independent of plaque formation.⁷

Transgenic mice with overexpression of human APP with V717F mutation and addition of the Swedish FAD mutation to the APP gene were studied. These researchers observed that the density of presynaptic terminals and neurones had decreased well before the FAD (V717F)-mutant human APP transgenic mice developed amyloid plaques. Electrophysiological recordings from the hippocampus revealed prominent deficits in synaptic transmission, which also preceded amyloid deposition by several months. Increased A β production in the context of the decreased overall APP expression, achieved by the double transgenic mice (DTG mice), further increased synaptic transmission deficits in the young mice without plaques.¹³ The result supports the new hypothesis that the soluble A β forms (protofibrils and small oligomers) could affect neurones, but escape detection by measurement of solid amyloid.¹⁴

PS Mutations

Genetic linkage studies mapped a locus associated with an aggressive early-onset AD to a series of polymorphic markers

located on chromosome 14q24.3. The actual disease gene (PS1) was subsequently isolated using a cloning strategy¹⁵ and a homologue (PS2) was then mapped to chromosome 1q42.1.^{16,17} To date, more than 70 different mutations have been discovered in the PS1 gene, the majority of these mutations are missense mutations, giving rise to the substitution of a single amino acid. There are 6 different mutations in the PS2 gene. In contrast to the high frequency of PS1 mutations, screening of large data sets revealed that PS2 mutations are probably rare.

To explore the functions of PS1 and PS2 genes, the best approach is a gene knockout strategy. PS1 deficient mice (PS1^{-/-} mice) die late in embryogenesis without evidence of AD. It has been proposed that this phenotype is a result of disturbed Notch signaling.^{18,19} PS1 is also involved in normal APP processing in neuronal cultures derived from PS1-deficient mouse embryos. PS1 mice have a defect in APP processing manifested by the failure of γ -secretase cleavage and the accumulation of c-terminal stubs of APP following α - and β -secretase cleavage. This indicated that PS1 is involved in γ -secretase activity.²⁰ This defect in APP processing is completely reversed by both wild-type and mutant PS1 genes. However, in contrast to PS1^{-/-} mice, PS2^{-/-} mice show no major developmental or APP processing defects.²¹

Accumulating studies showed that mutant PS1 and PS2 genes increase the production of amyloidogenic A β 42 and A β 43 peptides.²²⁻²⁵ Transgenic mice that express either wild type PS1 or A246E PS1 (Ala246→Glu mutation) were generated and mated with Mo/Hu-APPswe mice.²⁶ Similarly, Holcomb et al mated PS1 transgenic mice with APPswe transgenic mice (line Tg2576).²⁷ Both studies demonstrated that mice coexpressing mutant PS1 with APPswe developed A β deposits much earlier than age-matched animals that express APPswe alone, mutant PS1 alone, or wild-type PS1 with APPswe. In these transgenic models of A β amyloidogenesis, NFTs were not described. Moreover, neuronal loss is only observed in mice with a high amyloid burden.

β -Amyloid Precursor Protein Proteases

Although different subsets of AD phenotypic traits are reproduced in these transgenic models, none of them reproduce all the features typical of AD. Nevertheless, available

studies have provided evidence to support the view that A β 42 formation is an early and critical pathogenic event.²⁸

A β occurs in 2 predominant forms (A β 40 and A β 42) and is generated from the amyloid precursor protein by protein-cleaving (proteolytic) enzymes called β -secretase and γ -secretase.²⁹ AD-causing mutations in APP flank the protease cleavage site in APP and facilitate its cleavage. Those near the β -secretase cleavage site augment β -site proteolysis, leading to elevation of both A β 40 and A β 42; whereas mutations near the γ -site specifically increase production of A β 42. AD-causing mutations of presenilins modulate γ -secretase activity to enhance production of A β 42 (Figure 1)

β -secretase and γ -secretase were identified in 1999 and in 2000, respectively.³⁰⁻³⁴ β -secretase (beta site APP cleaving enzyme or BACE) is a new transmembrane aspartic protease. The gene for β -secretase (or BACE) is located on chromosome 11.³⁵ γ -Secretase is also a new transmembrane aspartyl protease. Accumulating evidence suggests that presenilins may be the catalytic component of γ -secretase, and may even be γ -secretase.³⁶ Li et al showed that PS1 (and PS2) contain the active site of γ -secretase, and transition-state-specific γ -secretase inhibitors specifically bind to the presenilins.³⁷ Li et al further showed that presenilin 1 is first synthesised as a zymogen (an inactive precursor protease), which does not bind to the inhibitor.³⁷

The β - and γ -secretase could be potential pharmacological targets. Blocking the 2 responsible proteases would be a reasonable approach for treatment interventions during the development of AD pathologies. Development of any medication requires validated animal models and well-defined molecular targets.³⁸

The above results could, perhaps, accelerate the development of a drug to stop or even reverse the neurodegenerative process. Intense efforts should be directed toward the development of clinically useful secretase inhibitors. Several

challenges remain. The main problem will be the delivery of such drugs to targets in the neurones through several formidable penetration hurdles, such as the blood-brain barrier and lipid bilayer of the subcellular space.

Another important problem is that these drugs should be highly selective: interference with other intracellular proteases and critical signalling pathways must be minimised. Further suitable experimental animal models will be needed to facilitate future studies.

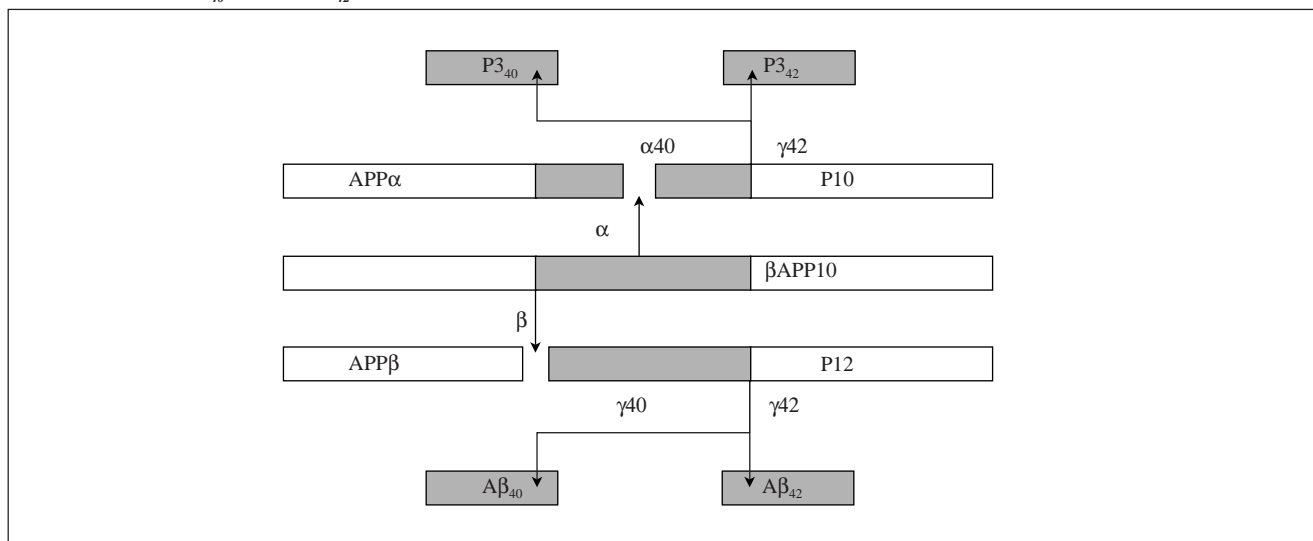
Apolipoprotein E Alleles

Apolipoprotein E (ApoE), the major serum protein involved in cholesterol storage, transport, and metabolism, is polymorphic and encoded by 3 alleles (apoE2, apoE3, and apoE4), which are located on chromosome 19q12-q13.^{39,40} ApoE3, the most common variant, reflects the presence of a cysteine at codon 112 and arginine at codon 158. ApoE4 reflects substitution of arginine for cysteine at codon 112. ApoE2 contains cysteine at codons 112 and 158. Numerous studies and data have confirmed the association between apoE4 and AD. The presence of 1 or 2 E4 alleles is associated with earlier onset of disease and enhanced amyloid burden in the brain.^{41,42} On the other hand, the E2 allele may be protective in this aspect.⁴³

Transgenic mice were created using human apolipoprotein E2, E3, E4 gene fragments driven by the human glial fibrillary acidic protein (GFAP) promoter. Smith et al utilised selected lines by breeding with apoE-deficient mice yielding mice which express the human transgenic apoE isoforms in the absence of endogenous apoE.⁴⁴ Aged apoE4 transgenic mouse brain fails to demonstrate any evidence of senile plaques.

However, APPV717F transgenic mice on a wild-type murine apoE show profuse amyloid deposition. In contrast, when the same transgene is expressed in an apoE^{-/-} background, there is only sparse, diffuse A β deposits.

Figure 1. Organisation of β -amyloid precursor proteins. α -secretase; β , β -secretase; γ , γ -secretase acting at the 40th amino acid of β -amyloid; 42, α -secretase acting at the 42nd amino acid of β -amyloid. Fragments of β -amyloid precursor proteins are soluble excluding β -amyloid_{40}}, β -amyloid_{42}} (Figure modified from Yan et al.³²)



The level of total A β and A β 42 in the hippocampus of APPV717F: apoE^{-/-} mice as measured by enzyme-linked immunosorbent assay is significantly less than that found in APPV717F: apoE^{+/+} mice. The ratio of total A β 42/A β , however, is not modified by the absence of apoE in this model. The effect of apoE on fibrillogenesis in vivo does not appear to act through alteration of A β 42-to-total A β ratio.

This finding and the fact that APP levels do not differ between animals with and without apoE suggest that apoE is influencing A β metabolism, structure, and/or clearance after being processed and released from APP.⁴⁵ Thus, it appears that the apoE allele type is not causative, but rather modulates the disease process.

To determine whether human apoE is also required for the development of fibrillar A β deposition and neuritic plaques in APPV717F transgenic mice, Holtzman et al bred APPV717F^{+/+}: apoE^{-/-} mice to apoE3 and apoE4 transgenic mice expressing human apoE isoforms under the control of the astrocyte-specific GFAP promoter.⁴⁶ A β deposition occurs in the presence of both apoE3 and apoE4. There is a significant isoform-specific difference in the amount of deposition, with greater A β deposition in apoE4 as compared with apoE3 expressing mice. These data show that apoE is required for amyloid formation with the FAD-mutant APP background and apoE4 has a greater effect on A β deposition than apoE3.

To further explore whether apoE4 simply does not function as well as apoE3 or whether it exerts detrimental effects that may interfere with the neuroprotective function of apoE3, Buttini et al analysed transgenic apoE knock-out mice that express apoE3 or apoE4 or both in the brain.⁴⁷ Apolipoprotein E3/E4 bigenic mice are as susceptible to neurodegeneration (by intraperitoneal injection of the glutamate receptor agonist, kainic acid) as apoE4 singly-transgenic mice (E4/0). Thus, apoE4 acts as a dominant negative factor that interferes with the beneficial function of apoE3. At 8 months of age, neurodegeneration is more severe in homozygous (E4/E4) than in hemizygous apoE4 (E4/0) mice.

This result indicates that the detrimental effects of apoE4 are dose-dependent. The exact mechanisms underlying the detrimental apoE4 activity remain to be determined. These experiments, consistent with other studies, strongly support that apoE4 acts as a genetic risk modifier for AD.

Alzheimer's Disease Vaccine: a Promising Therapy

These transgenic animal models can offer a viable means to test whether compounds can produce beneficial effects in an animal model prior to advancing such a drug into human trials. Schenk et al first reported that Alzheimer's-like pathology in mice could be halted by vaccination with solutions of A β peptide.⁴⁸ These researchers used PDAPP transgenic mice, which overexpresses human mutant APPV717F. The transgenic animals were immunised with A β 42 either before the onset of AD-type neuropathology

(at the age of 6 weeks) or at an old age (11 months), when β -amyloid deposition and other subsequent neuropathological changes were well established.

Some effects were noted — plaques were largely prevented from forming, and some of the pre-existing plaques in older mice even dissolved. Outcomes of Ab-plaque burden, neuritic dystrophy, and lissos were significantly improved by Ab42 treatment in both young and old animals.

In addition, the mechanism resulting in plaque reduction does not seem to produce any obvious signs of damage to the neuropil of Ab42-immunised animals. Histological examination of several organs, including the brain and kidney, revealed no signs of immune-mediated complications, despite the high levels of human APP expressed in these tissues and the significant antibody titre to endogenous mouse Ab peptide. These researchers observed that Ab42 immunisation resulted in the generation of anti-Ab antibodies and that Ab-immunoreactive monocytic/microglial cells appeared in the regions of remaining plaques.

They postulated that the possible mechanism of action is that anti-Ab antibodies facilitate clearance of β -amyloid either before deposition or after plaque formation by triggering monocytic/microglial cells to clear β -amyloid using signals mediated by Fc receptors.⁴⁸ This view seems to be in harmony with either the soluble Ab hypothesis or the amyloid cascade hypothesis. The antibodies derived from immunoreaction of soluble Ab may become useful tools for further identifying toxic peptides, and provide insights in future studies.

It remains unknown whether the vaccine could also stave off the cognitive losses that make Alzheimer's disease so devastating and whether the vaccine is safe enough to use to treat AD. Morgan et al tested the effects of A β vaccination in a different transgenic model (APPK670N, M671L + PS1M146L) in which the mice developed learning deficits as amyloid accumulated.⁴⁹

The experimental vaccine was prepared from human A β 1-42 (Bachem) and produced a sustained high titre level in the DTG mice. The control vaccine was prepared from keyhole limpet hemocyanin (KLH). The researchers tested mouse behaviour ('episodic-like' memory) in a radial arm water maze working memory task. The results showed that A β vaccination protects transgenic mice from developing memory deficits compared with KLH-immunised (control) transgenic mice. This vaccination-associated protection occurs in the presence of reduced, but still substantial, A β deposits. To address this finding, Morgan et al hypothesised that antibodies produced by vaccination either neutralise A β in a restricted neuronal compartment or deplete a non-deposited form of A β (for example, a soluble form).⁴⁹

Another laboratory found similar results that immunisation against A β (in β -pleated-sheet conformation) not only reduces plaque formation, but also ameliorates cognitive deficits in the TgCRND8 murine model of Alzheimer's disease that expresses a mutant (K670N/M671L + V717F) human APP₆₉₅ transgene.⁵⁰ These results strongly support

a causal link between a high level of A β and cognitive decline.⁵¹

Two groups suggest that either a small or a selective reduction in β -amyloid deposition may be sufficient to protect against dementia⁵² and both studies offer a promise regarding the safety and efficacy of vaccination with A β peptide vaccines. Potential applications in human subjects will require knowledge of the therapeutic mechanism and the specific antigens being targeted. Further intensive investigation will be needed to identify the best approach for safe and effective vaccine development.⁵³

Tau and Amyloid Plaques

Neurofibrillary tangles (NFTs) composed of the microtubule-associated protein tau are prominent in AD, Pick's disease, and other neurodegenerative diseases. Mutations in the gene encoding tau protein cause frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17). This demonstrates that there is a link between tau dysfunction and neurodegeneration. At least 11 missense mutations and a 3-base pair deletion (DeltaK 280) have been identified in exons 9 to 13. Additionally, 5 splice site mutations have been found in intron 10.⁵⁴ The most common mutation P301L (proline→leucine) derives from a C-to-T change in exon 10 on chromosome 17q21-22.^{55,56} However, no AD-causing tau mutations have been identified at present.

Gotz et al expressed human tau in P301L transgenic mice by using the neuron-specific mouse Thy1.2 promoter.⁵⁷ High expression of human P301L tau is obtained in cortical and hippocampal neurones in the mice. Accumulated tau is hyperphosphorylated and translocated from axonal to somatodendritic compartments and is accompanied by astrogliosis and neuronal apoptosis. As expected, P301L tau forms abnormal filaments. Neurofibrillary tangles occur in the cortex, brainstem, and spinal cord. The results showed that expression of the P301L mutation in mice causes neuronal lesions that are similar to those seen in human tauopathies. The conclusion is similar to that of Lewis.⁵⁸

There is a debate as to whether β -amyloid or tau is the primary cause of AD pathology and what is the pathophysiological relationship between them. Hardy et al proposed that the peptide A β 42 is central to the aetiology of AD and tau is produced either indirectly, by A β 42, or directly, in some form of frontotemporal dementia by mutations in tau itself.⁵⁹ Gotz et al injected synthetic A β 42 fibrils into the somatosensory cortex and hippocampus of 5- to 6-month-old P301L tau transgenic mice.⁶⁰ They observed 5-fold more NFTs in A β 42-injected animals than in animals injected with a control peptide.

NFT formation was found in the amygdala, remote from the injection site in hippocampus. Meanwhile Lewis et al crossed Tg2576 transgenic mice expressing the APPsw mutation (Lys670Asn, Met671Leu) with JNPL3 transgenic mice expressing mutant P301L 4-repeat tau and compared the pathology of the crossed mice with each of their parental lines.⁶¹ The resulting double mutant (tau/APP) progeny and the Tg2576 parental strain develops A β deposits at the

same age; however, relative to JNPL3 mice, the double mutants exhibit NFT pathology that is substantially enhanced in the limbic system and olfactory cortex. Both studies confirmed that A β 42 fibrils can significantly accelerate NFT formation in transgenic mice and revealed interaction pathologies in AD between APP or A β and tau.

Transgenic models reproducing the 2 pathological hallmarks of AD will help in the search for more effective therapies. The challenge for A β vaccination for AD treatment will be whether it can prevent NFT formation in animal models. If it is effective in retarding the formation of both amyloid plaques and NFTs in the transgenic models, the hypothesis of β -amyloid as a causative pathogenic factor in AD could be better established. AD will no longer be an unbeatable disease and finding therapeutic interventions will no longer be an impossible dream.

Non-hereditary Alzheimer's Disease Transgenic Animal Models

Non-hereditary AD transgenic animal models emerged during the past year. Capsoni et al succeeded in making anti-nerve growth factor transgenic mice.⁶² Nerve growth factor (NGF) is widely distributed in the basal forebrain cholinergic neurones (BFCNs) and in regions of the central nervous system innervated by the magnocellular BFCNs. NGF promotes the differentiation of BFCNs,⁶³ ameliorates lesion-induced abnormalities in these cells,⁶⁴ and reverses atrophy of BFCNs⁶⁵ and spatial memory impairments in aged rats.

Studies propose that NGF might be used as a potential therapeutic agent to prevent the degeneration of BFCNs in AD patients. However, the lack of suitable animal models in which the activity of NGF is chronically blocked in the adult central nervous system has not allowed proof of whether a reduced level and/or efficacy of NGF signalling may play a role in the pathogenesis of AD. The heterozygous NGF knockout mice (ngf^{+/-}) made by Chen et al showed shrinkage of basal forebrain and hippocampal neurones and a 20% reduction of ChAT-positive BFCNs associated with behavioural deficits.⁶⁶ However, the authors did not report any sign of AD-like neuropathology.

Capsoni et al used the neuroantibody technique to produce transgenic mice expressing a neutralising mAb (mAb aD11) directed against NGF, in which the levels of antibodies (a neutralising anti-NGF recombinant antibody) are 3 orders of magnitude higher in adult than in newborn mice.⁶² They reported that aged anti-NGF mice show massive and widespread neuronal loss, amyloid deposits, and extensive neurofibrillary pathology demonstrated with antitangle and antiphosphorylated tau antibody.

Moreover, these mice exhibit a severe cholinergic deficit in the basal forebrain and a behavioural impairment in retention and transfer of spatial memory tasks.^{62,67} Also, the progression of the neurodegeneration observed in anti-NGF mice showed a positive correlation with the severity of pathology, with the transentorhinal region showing the first signs of neurofibrillary lesions. Thus, the group considered that this phenotype is a comprehensive transgenic

model for AD research. Recent studies have shown the link between NGF and amyloid plaque, but the interaction mechanism is not clear.^{68,69}

NGF may be a potential therapeutic agent for the treatment of AD, but the problems of CNS delivery and side effects (particularly pain) still need to be resolved. Pharmacological approaches to enhance production of NGF in the CNS may also be useful in the treatment of AD.⁷⁰

Conclusion

In brief, much progress has been made towards understanding the aetiologies of AD in the past decade. Genetic models now available offer hope in the field of neuroscience. Knockout animals offer important clues to normal function. Transgenic animals not only allow investigation of the pathological role of mutant genes, but can also provide important models for drug development.⁷¹

Experimental data from AD transgenic mice models that reproduce both amyloid plaques and NFTs provide further support for the hypothesis that β -amyloid may be a causative pathogenic factor. The question of whether an increase in the level of $A\beta$ or the ensuing plaque formation is the causal factor in AD remains controversial. With the appearance of the soluble $A\beta$ hypothesis, transgenic animals with CNS deficits were once considered poor models of AD pathogenesis if they lacked amyloid deposits. At present, it appears that such animals provide good models for pathogenesis by non-fibrillar $A\beta$. These animal models reinforce the weak correlation between amyloid and disease in humans.¹⁵ Strategies that modulate the production, deposition, or toxicity of $A\beta$ might be considered reasonable therapeutic approaches. Currently, reduction of $A\beta$ levels is widely thought to be the most likely to succeed and means such as $A\beta$ vaccination and agents that inhibit $A\beta$ aggregation hold great promise.

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