



Modeling Alzheimer's disease in transgenic mice: effect of age and of Presenilin1 on amyloid biochemistry and pathology in APP/London mice

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Abstract

In transgenic mice that overexpress mutant Amyloid Precursor Protein [V717I], or APP/London (*APP/Lo*) (1999a. Early phenotypic changes in transgenic mice that overexpress different mutants of Amyloid Precursor Protein in brain. *J. Biol. Chem.* 274, 6483–6492; 1999b. Premature death in transgenic mice that overexpress mutant Amyloid precursor protein is preceded by severe neurodegeneration and apoptosis. *Neuroscience* 91, 819–830) the AD related phenotype of plaque and vascular amyloid pathology is late (12–15 months). This typical and diagnostic pathology is thereby dissociated in time from early symptoms (3–9 months) that include disturbed behavior, neophobia, aggression, glutamate excitotoxicity, defective cognition and decreased LTP. The *APP/Lo* transgenic mice are therefore a very interesting model to study early as well as late pathology, including the effect of age. In ageing *APP*Lo* mice, brain soluble and especially “insoluble” amyloid peptides dramatically increased, while normalized levels of secreted APPs α and APPs β , as well as cell-bound β -C-stubs, remained remarkably constant, indicating normal α - and β -secretase processing of APP. In double transgenic mice, i.e. *APP/Lo* \times *PS1*, clinical mutant *PS1[A246E]* but not wild-type human *PS1* increased A β , and plaques and vascular amyloid developed at age 6–9 months. The *PS1* mutant caused increasing A β 42 production, while ageing did not. Amyloid deposits are thus formed, not by overproduction of A β , but by lack of clearance and/or degradation in the brain of ageing *APP/Lo* transgenic mice. The clearance pathways of the cerebral amyloid peptides are therefore valuable targets for fundamental research and for therapeutic potential. Although hyper-phosphorylated protein *tau* was evident in swollen neurites around the amyloid plaques, neurofibrillary pathology is not observed and the “tangle” aspect of AD pathology is therefore still missing from all current transgenic “amyloid” models. Also the “*ApoE4*” risk for late onset AD remains a problem for

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modeling in transgenic mice. We have generated transgenic mice that overexpress human *ApoE4* (2000. Expression of Human Apolipoprotein E4 in neurons causes hyperphosphorylation of Protein *tau* in the brains of transgenic mice. *Am. J. Pathol.* 156 (3) 951–964) or human protein *tau* (1999. Prominent axonopathy in the brain and spinal cord of transgenic mice overexpressing four-repeat human *tau* protein. *Am. J. Pathol.* 155, 2153–2165) in their neurons. Both develop a similar although not identical axonopathy, with progressive degeneration of nerves and with muscle wasting resulting in motoric problems. Remarkably, *ApoE4* transgenic mice are, like the *tau* transgenic mice, characterized by progressive hyper-phosphorylation of protein *tau* also in motor neurons which explains the motoric defects. Further crossing with the *APP/Lo* transgenic mice is ongoing to yield “multiple” transgenic mouse strains to study new aspects of amyloid and *tau* pathology. © 2000 Elsevier Science Inc. All rights reserved.

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1. Introduction

Alzheimer’s disease (AD) is rapidly becoming a major social and medical problem due to and following the success of modern medicine and its effect on increased life expectancy. The special outcome of dementia, the term literally meaning “de-humanizing”, has dramatic and severe practical and psychological implications for the subjects and even more so for their relatives and caretakers. The loss of “self” and the complete dependence of the patient for all his essential needs is indeed “in-humane”. Early clinical diagnosis of AD is impossible and exclusion diagnosis without objective tests is problematic. The post-mortem pathological examination of the brain offers definitive diagnosis, essentially based on demonstrating intracellular neurofibrillary tangles and extracellular amyloid deposits as plaques, both visualized by silver impregnation of brain sections essentially as performed by Alzheimer in the beginning of the century.

Despite considerable progress in genetics and cell-biology and model-making, many problems remain about the precise definitions and mechanisms of neurodegeneration and the molecular and pathological components. Evidently, most crucial is to understand the precise mechanisms by which the different pathological lesions, i.e. amyloid and *tau*-pathology, originate and evolve (for reviews see Hardy, 1997; Selkoe, 1998). Genetic studies have contributed in a most decisive manner to fundamental studies of AD. Early onset familial AD (EOFAD) is inherited in a mendelian autosomal dominant manner and caused by mutations in the APP or Presenilin genes (Hardy, 1997). Although familial AD constitutes a minority of cases (<5%), their importance lies in their contribution to the identification of molecular links, providing the molecular basis for all subsequent fundamental research on APP and PS.

In the population of sporadic and late-onset AD cases (>65 years), over-representation of the $\epsilon 4$ allele of the *ApoE* gene remains the only genetic link. This is an indirect link which means that *ApoE4* carriers have an increased risk to develop dementia after 65. The absence of a direct causal relation makes that *ApoE* genotyping as no clear diagnostic value, although it identifies the largest fraction of elderly at risk. In these AD cases, the search for additional genetic components has produced many claims but no consistent candidate genes.

2. Pathology of amyloid and protein *tau* in AD brain

Significantly, despite the variable and divergent genetic causes, all AD patients develop comparable clinical symptoms and above all, present the same pathological lesions in their brain at autopsy, i.e. amyloid plaques and neurofibrillary tangles, mainly in hippocampus and cerebral cortex. Thus, regardless of the genetic cause all cases present common pathological parameters which constitute a major challenge for fundamental research. Some propose the amyloid plaques resulting from extracellular accumulated amyloid peptides as the most important while others favor neurofibrillary tangles (NFT), the intracellular aggregates of paired helical filaments (PHF) build of protein *tau* as major pathological culprits.

The fact that protein *tau* isolated from NFT from brain of AD patients is “hyper”-phosphorylated on serine and threonine in sequences of the type S/T-Proline, points to a deranged protein kinase-phosphatase balance. The phosphorylation sites are further established as epitopes for monoclonal antibodies, some defined as “AD-specific”, e.g. AT8 and Alz50 to name only these. In combination with biochemical approaches, proline-dependent *tau* kinases were eventually identified, such as GSK3 β and cdk5/p35. Whereas protein *tau* is involved in the dynamic stabilization of microtubuli as part of the axonal cytoskeleton, stabilizing and allowing axonal transport, it is surprising that mice rendered deficient in protein *tau*, display only minor physiological problems.

As opposed to APP and Presenilins, no genetic link has been found to the role of protein *tau* in AD. On the contrary, mutations in the *tau* gene (chromosome 17) cause types of dementia with a variable clinical picture, commonly known as Fronto-Temporal Dementia with Parkinsonism linked to chromosome 17 (FTDP-17). In the brain of these patients, neurofibrillary tangles develop similar to those in AD brain, but without amyloid plaques. This is important since it demonstrates that neurofibrillary tangles are more than a correlation and that protein *tau* itself can cause neurodegeneration and dementia. This corroborates the claims from pathologists that formation of intracellular tangles is an early aspect of AD and of primary pathological importance. The relation of AD and FTDP further identifies important regional differences in pathology and in the clinical outcome, which itself has to be considered in the strategy used to generate transgenic mouse models.

3. Questions asked in AD require experimental models: transgenic mice

Among the many and diverse molecular defects and brain lesions in AD patients that have been and are being reported, the major problem always remains what is “cause, correlation and consequence” not to mention the post-mortem artifacts. It is not known whether dementia and neurodegeneration are equal to loss of neurons or only to loss of functional synapses or even not more than excessive functional disturbances of synaptic transmission. Apoptosis of neurons or of synapses in the overall pathogenetic process remains an important issue for some whereby malfunction and excitotoxicity of the glutamate neurotransmitter system recurrently emerges as a hypothesis.

To understand fundamentally the biochemical, physical, cell-biological and physiological aspects and complexity of the brain, we need not only in vitro models but also

complex animal models, i.e. transgenic mice in which to implement the genetic causes to recapitulate the pathology of AD and invasive analysis. This article is not meant to produce an overview of the transgenic mice models that have been produced and published in the literature. On the contrary, we will explain our strategy and describe some of the salient features of the transgenic mice that we have produced and characterized in considerable depth now.

We are using a converging strategy aimed at incorporating the different molecular players that are known to be involved in AD from biochemical, genetical and epidemiological research, i.e. Amyloid Precursor Protein (APP), Presenilin1 (PS1), Apolipoprotein E (*ApoE4*) and protein *tau*. This strategy reflects the divergent causes as well as the convergent pathological characteristics that develop in the brain of AD patients. All transgenic mice that we are using in our studies are generated in the FVB genetic background.

The major and most informative models express the EOFAD APP/London mutant *APP695[V717I]* under control of the mouse *thy1*-gene promoter. This drives expression exclusively to the central neurons, starting only after birth, mainly in the second and third weeks excluding any major developmental effect of the transgene. All transgenic mice were derived by standard methods of superovulation and microinjection, as described (Moechars et al., 1996, 1999a). In all recombinant DNA constructs, the cDNA coding for the respective human proteins, wild-type or mutant, were ligated in the adapted mouse *thy-1* gene construct (Moechars et al., 1996) to replace the coding sequence and introns 2 and 3 of the mouse *thy1* gene but leaving the 5'-neuron specific control elements intact. The linearized mini-gene constructs were microinjected into pre-nuclear embryos from superovulated FVB/N females and transgenic founders identified by southern blotting of tail-biopt DNA by standard procedures. Transgenic strains that transmitted the transgene in a strictly mendelian fashion without integration-site effects, were compared and high expressing strains selected following western blotting of brain extracts. Double and triple transgenic mice overexpressing combinations of the respective human transgenes were obtained by standard cross-breeding, that all remain FVB and thus avoids all problems with genetic background when comparing single and multiple transgenic strains.

4. *APP/Lo* transgenic mice

Transgenic mice that overexpress wild-type or mutant APP recapitulate part of AD pathology, evidenced by the presence of amyloid plaques, cognitive deficits, behavioral deficits and other traits (Fig. 1) (Moechars et al., 1996, 1998a,b, 1999a,b; Games et al., 1995; Hsiao et al., 1996; Johnson-Wood et al., 1997; Sturchler-Pierrat et al., 1997). No model contains all aspects of AD pathology or all pathological lesions in the brain, with especially the formation of neurofibrillary tangles and its contribution to the pathology, if any, still lacking. More complete transgenic models can be expected to result from "multiple" transgenic lines, i.e. mouse strains that harbor different combination(s) of human genes. These are being obtained by cross-breeding available single transgenic mouse strains or mouse strains with null or conditionally modified genes. The strategy has already led to double transgenic mice with co-expression of human APP and Presenilin,



APP/Ld

EARLY PHENOTYPE

APP-PROCESSING:
INCREASED PRODUCTION
OF BETA-C-STUBS
OF BETA-A40/42

BEHAVIOR:
INCREASED AGGRESSION
NEOFOBIC REACTION
MEMORY AND LEARNING DEFICITS
(MORRIS WATER MAZE)

CELLULAR AND MOLECULAR:
DECREASED LTP
(LONG TERM POTENTIATION)
ALTERATIONS IN
GLUTAMATERGIC SYSTEM
(NMDA - KAINIC ACID)

LATE PHENOTYPE

PATHOLOGY:
ABUNDANT
AMYLOID PLAQUES
stained with anti-amyloid Abs
silversatining, thioflavinS

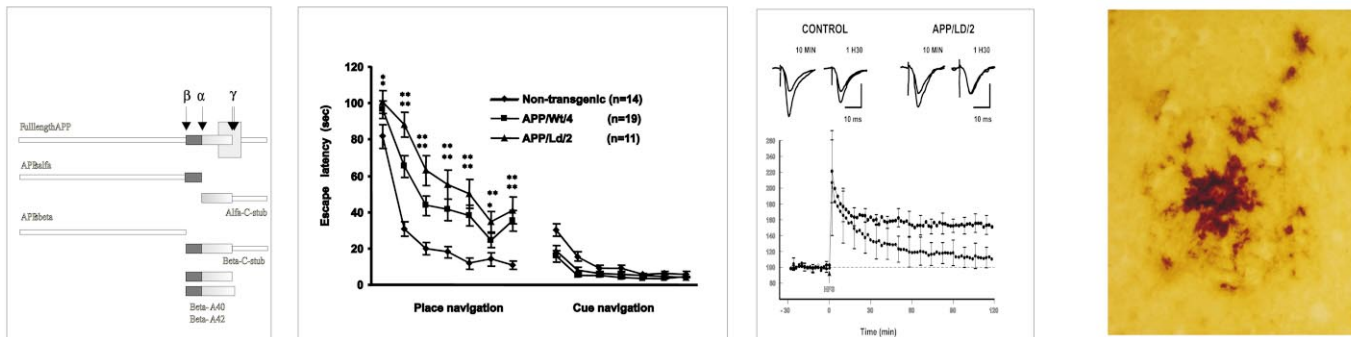


Fig. 1. Essential characteristics of the *APP/Lo* transgenic mice as model for Alzheimer's disease. The *APP/Lo* transgenic mice exhibit a remarkable combination of early clinical deficits or symptoms and late amyloid pathology (Moechars et al., 1999a,b). The diffuse and neuritic plaques and the vascular amyloid deposits (see Fig. 2) are in many aspects comparable or even identical to the diagnostic post-mortem pathology in the brain of AD patients (Van Dorpe et al., 2000). This is strongly in favor of our working-hypothesis that the effect of ageing as described here and elsewhere (Dewachter, 2000) as well as the early deficits (Moechars et al., 1998a,b, 1999a,b) are directly linked to and caused by the neuronal APP and amyloid peptide metabolism.

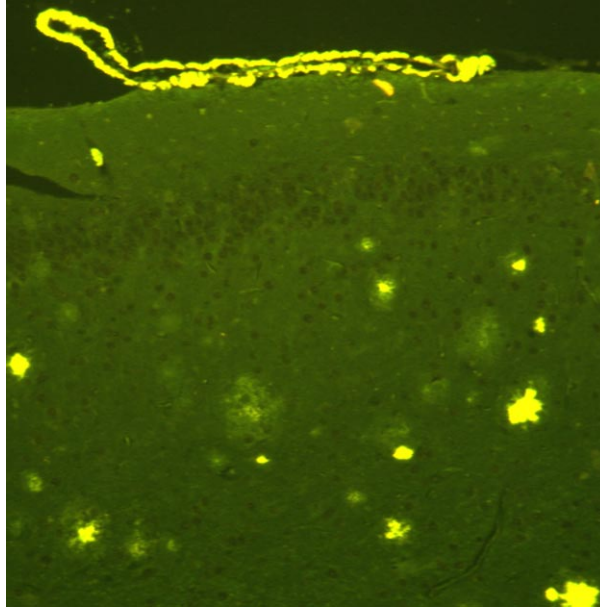


Fig. 2. Brain section of APP/Lo transgenic mouse at the age of 18 months, stained with thioflavinS to reveal the neuritic plaques, some diffuse plaques and a leptomeningeal blood-vessel loaded with amyloid. Note the strong staining of the amyloid-dense core of the many neuritic plaques and the cerebral angiopathy in the longitudinal cut blood vessel. Diffuse plaques do not stain efficiently with thioflavinS and their quantification following immunochemical staining demonstrate that they are much more abundant than neuritic plaques (Van Dorpe et al., 2000).

demonstrating essentially the increased production of A β 42 and earlier development of amyloid plaques, reminiscent of EOFAD (Borchelt et al., 1996, 1997; Citron et al., 1997; Holcomb et al., 1998; Dewachter, 2000; Van Dorpe et al., 2000).

Transgenic mice that specifically overexpress different wild-type and mutant isoforms of APP in their central neurons, displayed early phenotypic changes, including a marked cognitive impairment with decreased long term potentiation, differential glutamatergic responses, aggression and neophobia, among others (Moechars et al., 1999a,b, and references therein). In addition, the transgenic strain that expressed the highest levels of the APP/Lo clinical mutant, also developed a robust amyloidopathy in brain at the age of 12–15 months, involving both amyloid plaques and cerebrovascular amyloid angiopathy (Fig. 2) (Moechars et al., 1999a; Dewachter, 2000; Van Dorpe et al., 2000).

5. Effect of ageing

Ageing remains the most important and effective, but least understood parameter or risk-factor for dementia. We have therefore analyzed APP/Lo transgenic mice at the age of 3 months, at 6–9 months and at 15 months. We determined the effect of age on the level of

known intermediates and end-products of APP-processing, i.e. the membrane bound precursor (APPm), the soluble and plaque-associated amyloid peptides A β 40 and A β 42, the secreted ectodomain of APP processed by α -secretase (APPsa) and the C-terminal transmembrane and cytoplasmatic domain resulting from β -secretase cleavage, referred to as “ β -C-stubs” (Dewachter, 2000). This comparative analysis revealed that ageing did not appreciably affect the levels of either the α -secretase cleaved ectodomain nor the residual β -secretase cleaved C-terminal stubs of APP. Between the age of 3–15 months, the outspoken effect concerned the expected increase in soluble and insoluble amyloid peptides, A β 40 and A β 42, as well as an increase in the A β 42/40 ratio.

Thus, ageing of the *APP/Lo* transgenic mice caused precipitation of the amyloid peptides in the physical form of amyloid plaques, concomitant with or, more likely, following elevation of the levels of amyloid peptides, especially A β 42. Both phenomena did not occur before the age of 12–15 months in *APP/Lo* transgenic mice. These data confirm and extend findings obtained by others in two unrelated APP transgenic mouse models in which increased levels of A β peptides with age have been documented (Hsiao et al., 1996; Johnson-Wood et al., 1997). We have demonstrated that mRNA levels of both endogenous APP as well as of the APP transgene driven by the mouse thy1-gene promoter, increase with age in the brain of transgenic mice (Moechars et al., 1999b). Membrane-bound APP protein increased with age in the brain of *APP/Lo* transgenic mice in close parallel with mRNA levels (Dewachter, 2000). The concentration of APPm in brain of older mice relative to mice of 3 months, was increased to 122 and 169%, respectively, at 6–9 and 15 months. This appeared specific as it was not observed for the 85 kDa subunit of the Lipoprotein Receptor related protein (LRP) (Dewachter, 2000).

The combined data demonstrate that in brain of the *APP/Lo* transgenic mice, ageing per se did not cause a marked shift in the normal processing of APP as mediated by α - and β -secretase. Thus, the marked increase in both soluble and plaque-associated amyloid peptides, essentially situated between 12 and 15 months of age was not a direct consequence of a disturbed balance of the two main proteolytic events that govern APP metabolism.

6. *APP/Lo* \times *PS1* double transgenic mice

As explained above, single and double transgenic mice that co-express human *PS1* and APP, have proved to be informative for the mechanism of amyloid peptide formation in EOFAD. We generated double transgenic mice that co-express *APP/Lo* with wild-type or mutant human *PS1*. Only *PS1* mutant caused higher levels of amyloid peptides which precipitated and caused amyloid plaques to appear in brain already from the age of 6 months (Dewachter, 2000). The evident cause as demonstrated were the increased levels of A β 42, while A β 40 levels remained fairly constant. This resulted in a marked increased A β 42/40 ratio of soluble peptides: i.e. from 0.30 ± 0.04 to 0.96 ± 0.06 , respectively, in single *APP/Lo* and double *APP/Lo* \times *PS1* transgenic mice of 6–9 months. Insoluble or plaque-associated amyloid peptides increased even more dramatically, correlating with the much earlier development of amyloid plaques in the brain of the double transgenic

mice, i.e. at the age of 6–9 months versus 12–15 months in single *APP/Lo* transgenic mice (Van Dorpe et al., 2000).

7. Ageing and amyloid deposition

Surprisingly, since ageing did not shift the metabolism of *APP/Lo* from the non-amyloidogenic to the amyloidogenic pathway, but nevertheless caused increased levels of amyloid peptides and of course plaque and vascular amyloid. Since the extra incorporation of the mutant *PS1* in the double transgenic mice increased mainly the A β 42 levels in consistent with and expected effect exerted via γ -secretase, the combined data argument strongly against the hypothesis that the ageing effect acted via γ -secretase to increase production of amyloid peptides.

In addition, the absence of any evidence for decreased α -cleavage, other mechanism(s) need to be considered. We propose that accumulation of amyloid peptides results from their failing clearance, particularly of the A β 42 peptide. It is tempting to speculate that human genes to be discovered might be active in these unknown routes of clearance which might functionally connect to *ApoE4* and its receptor in brain, the LRP.

Finally, in the absence of indications of a major metabolic shift to the amyloidogenic pathway, ageing is proposed to act by decreasing the effective clearance of the amyloid peptides, particularly of A β 42 relative to the less amyloidogenic A β 40 peptide. This is an attractive hypothesis, as discussed, since effective removal of the amyloid peptides can involve or be mediated by many different intracellular and extracellular proteins, i.e. chaperones assisting or provoking secretion, recycling and adapter proteins for endosomal or other vesicles, cell-surface or secreted carriers, receptors or extracellular matrix proteins. Clearly, the genetic and epidemiological evidence, accrued over the last decade, for the involvement or association of different genes, encoding a functional wide variety of proteins, in sporadic or late onset AD, would find a very plausible explanation in this hypothesis.

8. *ApoE4* and *tau* transgenic mice

Whereas work on APP and PS transgenic mice is most advanced, we have generated transgenic mice that overexpress human protein *tau* or human *ApoE4* in their central neurons, using the same strategy as outlined above. These transgenic mice are briefly discussed here and together, because rather surprisingly both develop a pathological phenotype that includes severe axonopathy and axonal degeneration, leading to motoric problems.

Protein *tau* transgenic mice were psychomotorically impaired and developed prominent axonopathy in brain and spinal cord (Spittaels et al., 1999). Typical axonal dilations in brain and spinal cord develop by accumulations of neurofilaments, mitochondria and diverse types of vesicles. This clearly suggested that higher than normal levels of protein *tau* caused defective axonal transport, which in turn caused the accumulated material in the dilatations and, distal to these, the axons degenerate and impaired muscular innervation. The human protein *tau* is hyper-phosphorylated progressively in ageing transgenic

mice, as evidenced by reaction with specified monoclonal antibodies, i.e. AT8 and Alz50 among others. Nevertheless, intraneuronal fibrils or tangles were not observed demonstrating that merely increasing the concentration of protein *tau* is sufficient to cause neuronal injury (Spittaels et al., 1999).

The mechanism by which *ApoE4* contributes to the development of neurodegeneration remains unknown. To test one specific mode of action of *ApoE*, we have generated transgenic mice that overexpress human *ApoE4* in different cell-types in the brain using four distinct gene promoter constructs. Overexpression of human *ApoE4* by way of the *thy1*-gene promoter in neurons of transgenic mice, produced mice with a severe phenotype as opposed to overexpression by the GFAP promoter, which was innocuous even till the age of 20 months (Tesseur et al., 2000). The *thy1-ApoE4* transgenic mice developed severe motoric problems from the age of 3 months accompanied by muscle wasting, loss of body weight and premature death. Since this resembled in several aspects the pathology in the *tau* transgenic mice described above, we analyzed endogenous mouse *tau* protein for hyperphosphorylation. In three independent transgenic lines, increased phosphorylation of protein *tau* was correlated with neuronal *ApoE4* expression levels (Tesseur et al., 2000). Hyperphosphorylation of protein *tau* increased with age, in parallel with astrogliosis and ubiquitin positive inclusions in hippocampal neurons. Although unexpected, the findings that neuronal expression of *ApoE* can increase hyperphosphorylation of protein *tau*, offers a potential model for the role of *ApoE* in AD.

As a final step, we are generating different types of compounded multiple transgenic mouse strains to allow us to define and study the synergistic actions that are expected to operate *in vivo*, between the major players that are known to be involved in AD, i.e. APP, *PS1*, protein *tau* and *ApoE4*. At this moment, the “tangle” aspect of AD pathology is still missing in any transgenic mice model, as opposed to “plaque and vascular amyloid” preventing definition of its importance in the overall pathology of AD — early or late. From the results published and briefly discussed, it is becoming evident that implementation of the *tau* pathology would appear not to be straightforward by simple overexpression and increasing its neuronal levels. Understanding the role of hyper-phosphorylation of protein *tau* and its precise role and impact in the overall pathology of AD, if any, will require fundamental insight in diverse aspects of cellular signaling pathways, which appears now to include even a novel aspect or effect of *ApoE4* expression (Tesseur et al., 2000).

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