
I. Torres-Aleman

Laboratory of Neuroendocrinology, Cajal Institute, Consejo Superior de Investigaciones Científicas and Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), 28002 Madrid, Spain

It is lay knowledge now that Alzheimer’s dementia (AD) is one of the most devastating diseases afflicting our societies. A major thrust in search for a cure has relied in the development of animal models of the disease. Thanks to progress in the genetics of the rare inherited forms of AD, various transgenic mouse models harboring human mutated proteins were developed, yielding very significant advancements in the understanding of pathological pathways. Although these models led to testing many different new therapies, none of the preclinical successes have translated yet into much needed therapeutic improvements. Further insight into the metabolic disturbances that are probably associated with the onset of the disease may also rely on new animal models of AD involving insulin/IGF-I signaling that could mimic the far most common sporadic forms of AD associated with old age. Combination of models of familial AD that develop severe amyloidosis with those displaying defects in insulin/IGF-I signaling may help clarify the link between putative initial metabolic disturbances and mechanisms of pathological progression. (Endocrinology 149: 5952–5957, 2008)

A MAJOR GOAL in biomedicine is to obtain useful experimental models of human diseases. Naturally, in vivo models mimicking all the clinical traits of the target disease are the ideal setting. Most available models of human disease do not reach this golden standard, but for several important diseases, a reasonably good approximation has been achieved. This is the case for transgenic mouse models of Alzheimer’s dementia (AD), one of the main causes of disability and death in the elderly (1). Current Alzheimer models rely on information gathered from inherited forms of the disease, far less common than sporadic ones but indistinguishable from the clinical point of view. Thus, both early familiar and late-onset sporadic forms progressively develop cognitive deterioration that in early stages affect memory systems and at late phases impair all cognitive spheres of daily life activities (2).

Similarly, the underlying neuropathology in the two forms of the disease include extracellular deposits of β-amyloid (Aβ) peptides, intraneuronal accumulation of aberrant forms of hyperphosphorylated tau, a microtubule-associated protein, forming neurofibrillary tangles, and synapse and neuronal loss. Many other pathological changes are present in AD brains, but amyloid plaques and tangles, as originally defined by Alzheimer (3), are universally considered the traits that characterize the disease. Although the etiology of AD remains unknown, these two characteristics are used for the only established diagnosis of the disease presently available that relies on postmortem verification. Identification in various human pedigrees of familial AD of mutations on the amyloid precursor protein (APP), the transmembrane protein giving rise to Aβ (4), and on presenilin-1 and -2, part of the membrane enzymes involved in APP processing (5), soon led to the production of transgenic mice expressing these human proteins and to many other genetically modified models including ablation, knock-in, and compound genotypes of these proteins (6).

The availability of these mouse models originated a wealth of data on pathological mechanisms in AD. It is fair to say that the majority of advances in AD have been made possible by these models (7). Another very profitable use of these mice has been to test new therapies including not only a diversity of drugs but also vaccines or behavioral strategies. However, in this case, the information obtained has met with somewhat mixed success. This may be exemplified by the significant improvement observed in transgenic mice receiving various forms of Aβ vaccines that turned into a clinical assay that had to be halted due to unexpected lethal effects (meningoencephalitis) of the therapy (8). Although these tough lessons will hopefully translate into better designed and safer clinical assays, including new vaccine forms (9), the failure of these animal models to yield not a single reliable clinical outcome has recently put into question their utility as preclinical models of sporadic AD. Not coincidentally, among many other new proposals, a novel potential pathogenic route involving IGF-I, which in turn encompasses a former one that postulated impaired insulin function as a major cause of sporadic AD, has recently gained attention (10).

In this review, I will summarize the main animal models of AD based on genetic manipulation and describe the advancements that originated and their preclinical use. The advent of new animal models based on a different etiopathogenic postulate involving metabolic disturbances in the brains of AD patients will also be discussed and integrated within current concepts of the causes of the disease.

Transgenic Mouse Models of AD

Transgenic mouse models harboring mutations found in familial AD constitute the most widely used in vivo approach
to study this disease. Few years elapsed between the description of a dominant missense APP mutation in familial AD (11) and the generation of the first transgenic mouse bearing a mutated human APP (12) that effectively mimicked several AD features, with prior attempts with wild-type APP made earlier (13). This testifies to the fast pace of this dynamic field. Indeed, the number of transgenic models of AD bearing human mutations is now so large and the literature produced so vast that only the major findings can be summarized. A detailed account of the different mouse models and their phenotypes may be found in various excellent reviews (6, 14). Although invertebrate models have also been developed, because they do not constitute the mainstream of research in AD models, they will not be further considered.

Probably the main observation arising from mouse models was that excess Aβ elicits cognitive impairment even in the absence of neurofibrillary tangles or neuronal loss (7). This finding posed this pathological change as the main culprit and therefore substantiated the amyloid cascade hypothesis of AD that favors brain amyloidosis as the cause of the disease (15). Consequently, the pathogenic contribution of neurofibrillary tangles (16) was neglected. Later observations combining various transgenic models concluded that the extent of Aβ plaque load does not correlate with cognitive loss. This triggered a subsequent refinement of the amyloid hypothesis that stipulated that soluble amyloid oligomers rather than insoluble deposits are responsible for synapse loss underlying the pathology (17). Presenilins are key components of the γ-secretase complex that cleaves APP and other transmembrane proteins (18). Most familial forms of AD present with mutations in either presenilin-1 or -2 (PS1 or PS2), which appear to result in enhanced Aβ production (18). Although transgenic mice bearing human presenilin mutations do not develop AD-like features unless they are combined with APP mutations (14), the double conditional PS1/PS2 mutant in adult brain provides the only mouse model of familial AD that includes both cognitive loss and neurodegeneration (19). Currently, double transgenics expressing mutant APP and either of the mutated presenilins (PS1 or PS2) are the most widely used models. The reason is that two main features of the disease, amyloidosis and cognitive loss, develop very rapidly in these mice, making them better suited for experimental studies (20).

Because none of the above models developed neurofibrillary tangles, the main neuropathological disturbance in AD together with amyloid plaques, mouse models expressing human tau mutations were generated. These transgenic mice did develop tangles. Most significantly, they also showed cognitive disturbances together with neuronal loss (21). The latter trait is characteristic of human AD but absent in amyloid models. However, these models lacked amyloid plaques. In turn, combined expression of mutated forms of tau with mutant APP provided the first AD models displaying the three main characteristics of the disease, intracellular neurofibrillary tangles (tauopathy) together with cognitive loss and amyloid plaques (22). These models have been extensively used to dissect out the pathogenic link between amyloidosis and tauopathy, with no definitive conclusions reached yet. However, a main conceptual drawback of these latter models is that no mutations in tau have so far been detected in familial AD. Rather, the mutated tau forms employed in these transgenic mice are found in frontotemporal dementia, an inherited neurodegeneration that lacks amyloid deposits (23). Nevertheless, because tauopathy correlates well with cognitive loss, a key role for this pathological derangement is supported (23).

Together with a better mechanistic insight into the neuropathology of AD, transgenic models provided a powerful preclinical tool for new treatments. Unfortunately, and in contradistinction to the great gain in pathogenic knowledge, new treatments arising from this knowledge have not followed the same fast pace of achievement (24). For instance, the membrane protease β-secretase (BACE) was first described as a potential target in AD almost 10 yr ago, but no successful treatment has yet arisen from this information (25), and its utility as a drug target is now questioned (26). Again, the list of potentially therapeutic compounds and treatments tested in transgenic mice is so extensive that only a brief summary of major conclusions is possible (see Ref. 27 for an update). Drugs used in preclinical tests fall into either disease-modifying or symptomatic categories. The former derived mostly from new therapeutic targets unveiled in part with transgenic models. For instance, detailed knowledge of the routes leading to Aβ production provided numerous new druggable targets (28) aiming to reduce Aβ production in different manners. Additional approaches not focusing on amyloid reduction are also wisely encouraged (29). Development of symptomatic drugs, which are becoming less supported lately (30), derived from the relative success of acetylcholinesterase inhibitors, the main marketed drugs in AD that aimed to correct behavioral deficits. These drugs were developed without a firm experimental/conceptual basis. Currently, it is unlikely that new symptomatic drugs will be approved as regulatory agencies seek robust biological surrogates of new therapies.

Beyond the Amyloid Cascade Hypothesis: Metabolic Disturbances in AD and Corresponding Animal Models

Despite the unquestionable benefit that these genetic models have provided to a better understanding of pathogenic processes in AD, we still have more questions than answers of underlying pathogenic mechanisms. Probably the major drawback of transgenic models is that very few of them mimic extensive neuronal loss typical of AD. And those few are not based in actual pathological changes seen in AD patients. In addition, none of the available models have helped to reach a comprehensive explanation of the origin of the disease. That is, amyloidosis is clearly a key component in AD, but its trigger, increased production, decreased degradation, or faulty clearance, or a combination of these, and its role as a causative agent in sporadic AD remains undefined. For this reason, new hypotheses of the origin of the disease and their corresponding animal models are blooming. For instance, loss of function of presenilins have been claimed to explain both familiar and sporadic forms of AD (18). The pathogenic contribution of the e4 isoform of apolipoprotein E and its relation to cholesterol metabolism in late-onset forms is also actively studied (31), together with
the role of cholesterol in the etiology of AD (32). Other processes such as calcium dysregulation, disturbed autophagy, aberrant proteasome function, mitochondrial failure, and many other alterations have been proposed as progression rather than causative factors in the pathological cascade.

A candidate etiological event in AD is impaired energy balance in brain cells. Metabolic disturbances associated with AD are becoming a subject of intense scrutiny because regional deficits in glucose use are firmly established in AD brains. As a matter of fact, brain imaging of glucose consumption is probably the most reliable imaging-based diagnosis of the disease at present (33). Early observations indicated a high prevalence of insulin resistance in AD (reviewed in Ref. 34), which was recently postulated as an early prognosis marker of the disease (35). Although still controversial (36), type 2 diabetes has also been proposed to increase the risk of AD (37), whereas altered glucose metabolism may be an early sign of cognitive decline (38), and systemic levels of insulin correlate with AD neuropathology (39). Many other observations support a role of insulin dysregulation in AD, as highlighted by the type 3 diabetes label recently assigned to the disease (40). The underlying notion is that loss of insulin function is the cause of the disease because insulin is a key regulator of cell metabolism and is involved in higher brain functions including cognition (41). Several animal models reinforce this possibility. Thus, defects in insulin signaling in IRS-2 knockouts present tau hyperphosphorylation in their brains (42), a defect also observed in streptozotocin-diabetic animals (43). Moreover, brain-specific deletion of the insulin receptor originated the same pathological change in tau phosphorylation (44). A practical outcome of these observations has been to test insulin and insulin sensitizers (peroxisome proliferator-activated receptor agonists) in AD patients. Initial results are encouraging, but more detailed clinical studies are warranted (45, 46).

However, deficits in insulin signaling do not fully model AD pathology (44). Consequently, additional or alternative causes are sought. For instance, estrogen deficiency with advancing age in women, leading to impaired mitochondrial energy output, has been proposed as a key contributor to metabolic imbalance in AD (47). This may help explain gender differences in disease incidence and support a possible use of steroid hormone therapy (48). An additional altered process may be IGF-I signaling upstream of insulin dysregulation because IGF-I and insulin functions are closely intertwined. While examining the action of IGF-I at the blood-brain barrier (49), a link between circulating IGF-I and brain amyloid was observed (50). Based on these findings, we hypothesized that disturbances in IGF-I precede altered brain insulin actions (51). A detailed account of this hierarchical relationship can be found elsewhere (52). In essence, disrupted signaling of systemic IGF-I into the brain (probably initiated at the blood-brain barrier as a consequence of interactions between genetic background and environmental factors) would lead to brain insulin dysregulation and neuropathological changes typical of AD (Fig. 1).

![Figure 1](https://example.com/figure1.png)

**FIG. 1.** Disrupted systemic IGF-I input to the brain may arise from a combined deficiency/resistance associated with 1) old age, 2) IGF-I/IGF-I receptor polymorphisms, 3) various illnesses, or 4) lifestyle factors such as sedentary life, imbalanced diet, or stress. In turn, this originates brain insulin-deficient signaling. Together with disturbed IGF-I input, reduced brain insulin function may lead to all pathological derangements associated with AD that are classically attributed to amyloidosis in the amyloid cascade hypothesis (81).
an additional compounded pathway may lead to impaired addition, if indeed IGF-I receptor levels are increased in AD tion will lead to altered insulin receptor signaling (59). In IGF-I may act as an insulin receptor cofactor, and its reduc- ional alternative, nonexclusive explanations are possible. the majority of the neuroprotective pathways where IGF-I is involved appear altered in AD (Table 1). Moreover, an animal model based on this proposal mimicked late-onset AD in rodents that included cognitive loss, tauopathy, and increased Aβ levels (53). This hypothesis predicted early increases in serum IGF-I levels in AD patients (51), as recently reported (54), together with a gradual decline at later phases of the disease, also recently observed in AD (55). This pattern may reflect a progressive process of resistance/deficiency of IGF-I input to the brain (51, 56), also recently claimed to be observed in AD brains (57) and reminiscent of the pathological progressive insulin resistance/deficiency in type 2 diabetes. When combined with classical models of AD the corresponding animal model helped to clarify the relative contribution of plaque load and amyloid clearance to cognitive derangement because enhanced clearance was sufficient to ameliorate cognitive loss without any effect on plaque load (58). This supports the emerging notion that even in the absence of amyloid plaque-clearing effects, cogni- tive improvement is possible.

This theoretical proposal provides a conceptual frame to place findings obtained from the corresponding animal models. Thus, disrupted brain insulin signaling will lead to most but not all AD pathology because a concomitant lesion in IGF-I signaling is required. As the status of insulin and insulin receptor levels in AD brain is controversial (57), a key aspect of this proposal would be to determine the existence of a pathway linking disrupted IGF-I signaling to reduced insulin action. Although entirely speculative at present, various alternative, nonexclusive explanations are possible. IGF-I may act as an insulin receptor cofactor, and its reduc- tion will lead to altered insulin receptor signaling (59). In addition, if indeed IGF-I receptor levels are increased in AD (57), a possible resultant increase in IGF-I/insulin receptor hybrids will originate loss of insulin action (60). Conversely, an additional compounded pathway may lead to impaired insulin action. First, reduced IGF-I input will lead to amyloid- osis due to imbalanced Aβ metabolism (53, 61). Then, reduced availability of insulin-degrading enzyme and impaired insulin binding to its receptor will follow (62, 63). In addition, low insulin-degrading enzyme levels will also contribute to insulin resistance (64). Once IGF-I and insulin functions in the brain are compromised, all pathological disturbances in AD will ensue through processes such as oxidative stress or inflammation, as discussed in detail elsewhere (51).

Notably, many observations combining metabolic and hormonal manipulations in classical transgenic mouse models of AD conform to this proposal. For instance, the observed improved cognition and pathology after exercise or environmental enrichment in mutant APP mice (65, 66) can be potentially explained by enhanced brain IGF-I input under these conditions (49, 67). Conversely, the aggravation of AD symptoms in transgenic mice fed with imbalanced diets (68, 69), or the beneficial effects of healthy diets (70, 71) explained by corresponding changes in insulin signaling in brain, can also be secondary to initial changes in brain IGF-I input (72). New proposed links between APP and glucose metabolism and growth (73), or the higher risks to suffer AD in obese subjects (74), add fuel to this argument. Furthermore, the purported role of ovarian hormones in development of AD, as tested in various transgenic models (75, 76), could also be linked to disturbances in IGF-I signaling because this growth factor is intimately linked to gonadal function (77) and also declines with age.

In conclusion, as more work is needed to establish the role of metabolic disturbances in the etiology of AD, the combined use of traditional transgenic mouse models with metabolic imbalances constitute a reasonable approach to gain further insight into these processes.

Acknowledgments

Received June 17, 2008. Accepted July 29, 2008.

Address all correspondence and requests for reprints to: Ignacio Torres-Aleman, Cajal Institute, Avenida Dr. Arce 37, 28002 Madrid, Spain. E-mail: torres@cajal.csic.es.

This work was funded by the Spanish Ministry of Education and Science (SAF2007-60051), Spanish Ministry of Health (CIBERNED), and the Madrid Local Government (CAM NEURODEG MODELS Program).

References


46. Szabo R, Schaffer L, Laustrup-Larsen I, Andersen AS, Shaw AC, Mathiasen IS, Brandt L 2006 Hybrid receptors formed by insulin receptor (IR) and insulin-like growth factor I receptor (IGF-IR) have low insulin and high IGF-1 affinity irrespective of the IR splice variant. J Biol Chem 281:25869–25874


48. Llorente RE, de Tulio M, Alonso LG, Leissring MA, Kaufman SB, Roher AE, Torres-Aleman • Minireview

Endocrinology is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.