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## *Review*

# APP transgenic mice and their application to drug discovery

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**Summary.** The development of transgenic mice expressing mutated forms of the human amyloid precursor protein (APP) and presenilin-1 (PS1), proteins associated with familial forms of Alzheimer's disease (AD), has provided a backbone for translational studies of potential novel drug therapies. Such mice model some aspects of AD pathology in that they develop senile plaque-like deposits of the amyloid beta-protein (Aß) together with inflammatory pathology and some degree of neurodegeneration. Aß deposition is considered to be a potentially pathogenic feature of AD and drug discovery programmes utilising such mice and associated with drugs now reaching the clinic have been largely directed towards decreasing the deposition. This goal has been achieved in the mouse models, although the agents developed have not, to date, shown evidence of efficacy in AD sufferers and, in some cases, have worsened the clinical state. Nevertheless, reducing the pathological features of the disease continues to be the objective of pharmacological intervention and ongoing programmes continue to use transgenic mice expressing mutated APP and PS1 transgenes in attempts to overcome issues and difficulties arising from the initial clinical trials and to explore new approaches to AD treatment.

**Keywords:** Alzheimer's disease, Neurodegenerative pathology, Drug discovery

## 1. Introduction

The presence of the fairly distinctive pathology of senile plaques and neurofibrillary tangles in the brains of Alzheimer's disease (AD) sufferers points to one or both of these features being implicated in the aetiology of the disease. Analysis of plaques and tangles shows them to be comprised largely of the amyloid beta-protein (Aß) and hyperphosphorylated tau, respectively. In both pathologies the proteins appear to be highly aggregated and resistant to degradation. The majority of cases of AD arise spontaneously, although in a few, a genetic component is apparent where there is a family history of the disease, usually with an earlier age of onset than in the sporadic cases. These familial forms of the disease have been linked, largely, to mutations in two proteins, the amyloid precursor protein (APP) and presenilin-1 (PS1). APP is cleaved by ß-secretase (BACE-1, beta-site APP cleaving enzyme) (Hussain et al., 1999; Sinha et al., 1999; Vassar et al., 1999; Yan et al., 1999) and gamma secretase (De Strooper et al., 1999; Wolfe et al., 1999), a component of which is the protein presenilin-1. Thus, two proteins intimately involved in the production of Aß are known to be mutated in differing familial forms of AD (Chartier-Harlin et al., 1991; Goate et al., 1991; Sherrington et al., 1995). In addition, in Down syndrome, where there is an extra copy of chromosome 21 (the chromosome bearing APP), AD-like symptoms and pathology emerge as the sufferers age (Iwatsubo et al., 1995). Following acute head injury (Roberts et al., 1994) and in dementia pugilistica (Roberts et al., 1990), where chronic brain injury may have resulted from participation in the sport of boxing, the dementia is known to be associated, from post-mortem studies, with senile plaques. Although mutations in tau protein or in enzymes involved in its catabolism are not a feature in familial forms of AD, nevertheless, neurofibrillary tangles composed of hyperphosphorylated tau are found in Down syndrome and dementia pugilistica, as well as in AD. Taken together, the above evidence, together with data showing neurotoxic properties for Aß, both *in vitro* and *in vivo,* led to the proposal of the Amyloid Hypothesis of AD pathogenesis in 1991 (Hardy and Allsop 1991).

Having laid down the fabric of a causal relationship between Aß production and disease, the search for

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therapeutic agents capable of interfering with Aß production began and the need for an animal model of AD became apparent. The history of AD model development, particularly the generation of APP transgenic mice bearing familial AD mutations, has been reviewed extensively (eg. Howlett and Richardson 2009). Although such mice invariably show a number of pathological features of AD, a major question has been whether they are true or useful models of the disease state or simply reflect an artificial state arising from the gross over-expression of amyloid protein. This present review examines whether existing models have provided any benefits to drug discovery and looks at recent developments in our understanding of APP transgenic mouse pathology with a view to the development of more appropriate models of the AD. The major lines of APP transgenic mice referred to in this review are described in Table 1.

## 2. From translational studies to clinic

### *i) Clinical trials of putative AD therapeutics*

A starting point to consider the utility of AD animals is to look at what has gone before. Putative AD disease modifiers reaching the clinic in the last few years are a product of translational studies undertaken utilising the animal models developed a decade or more ago. One of the earliest studies in transgenic mice demonstrated that vaccination with the Aß peptide offered a potential therapeutic approach capable of preventing or removing one of the main pathological features of AD (Schenk et al., 1999). The first trial of this amyloid vaccination strategy (AN-1792, Elan Pharmaceuticals), although halted for safety reasons (unacceptable incidence of aseptic meningoencephalitis possibly associated with an inappropriate T-cell response to the adjuvant used (Senior, 2002; Nicoll et al., 2003)) did provide some indication of proof of efficacy, at least in terms of plaque removal. Where possible, subjects were followed through to eventual post-mortem, up to six years later (Holmes et al., 2008). In this follow-up analysis, significant decreases in Aß plaque pathology were apparent. These changes, however, were not accompanied by any improvement in clinical state, ie. clinical data did not offer support for the Amyloid Hypothesis of AD pathogenesis.

Subsequently, a considerable effort has been directed towards overcoming the clinical issues associated with the AN1792 trial, particularly in attempting to deal with what was seen as an inappropriate cellular autoimmune response and/or possibly an issue arising from the adjuvant employed. Further clinical trials (FDA Phase IIa/b) of a small Aß1-6 fragment attached to a carrier protein and employing an alternative saponin adjuvant are currently ongoing (ACC-001, Elan/Wyeth: http://www.alzforum.org/drg/drc/detail.asp?id=102; last accessed 01 July 2011).

Passive immunisation approaches have employed the administration of antibodies raised to various Aß fragments. A range of such antibodies have been shown to reduce Aß load in the brains of PDAPP mice (DeMattos et al., 2001; Dodart et al., 2002). These studies have led to the development of putative vaccines such as bapineuzumab and solanezumab that are humanised monoclonal antibodies targeted against the N-terminal and mid-regions of Aß, respectively; a large scale phase III trial of bapineuzimab in mild to moderate AD is currently ongoing (http://clinicaltrials.gov/ct2/show/NCT00574132; last accessed 01 July 2011). Early stage trials of bapineuzumab have shown limited efficacy and some safety issues have been apparent (http://newsroom.elan.com/phoenix.zhtml?c=88326&p=irol-newsArticle&ID=1272546&highlight= ; last accessed 01 July 2011).

Inhibiting the amyloidogenic cleavage of APP would appear to be a legitimate target for an Aß lowering agent. Indeed, considerable effort has been directed towards the inhibition of the enzyme BACE1, responsible for the cleavage at the N-terminus of Aß, and the gamma

**Table 1.** Transgenic mice referred to in this review.

| Mouse line | APP | Promoter | APP mutation | PS1 (and tau) mutation | |
|---|---|---|---|---|---|
| PDAPP | Minigene | PDGF | V717F (Indiana) | none | Games et al., 1995 |
| Tg2576 | 695 | Hamster prion | KM670/671NL (Swedish) | none | Hsiao et al., 1996 |
| APP23 | 751 | Thy-1 | KM670/671NL | none | Sturchler Pierrat et al., 1997 |
| TgCRND8 | 695 | Hamster prion | KM670/671NL+V717F | none | Chishti et al., 2001 |
| J20 | Minigene | PDGF | KM670/671NL+V717F | none | Mucke et al., 2000 |
| TASTPM | 695 | Thy-1 | KM670/671NL | M146V | Howlett et al., 2004 |
| ArcSwe | 770 | Murine prion | KM670/671NL+E693G (Arctic) | none | Philipson et al., 2009 |
| APPswe.PS1dE9 | 695 | Murine prion | KM670/671NL | Exon 9 del. | Jankowsky et al., 2004 |
| APP.PS1.KI | 751 | Murine thy-1, murine PS1 | KM670/671NL+V717I (London) | M233T, L235P | Casas et al., 2004 |
| 3xTg-AD | 695 | Murine thy-1 | KM670/671NL | M146V+tau.P301L | Oddo et al., 2003 |
| 5xFAD | 695 | Murine thy-1 | KM670/671NL+I716V+V717I | M146L, L286V | Oakley et al., 2006 |

APP mutations refer to 770 numbering. Other APP mutations utilised include E693Q, E693deletion, D694N. Other PS1 mutations include L166P, L235T, A246E.

secretase complex, the actions of which at the C-terminus of Aß results in the release of the Aß peptide from its precursor protein.

The intraperitoneal injection of the BACE inhibitor CTS-21166 (CoMentis) in APP.Swe + Lon mice (ie. transgenic mice expressing APP with Swedish and London FAD mutations) reduced brain Aß40 and Aß42 hippocampal/cortical plaque load by 40% (http://www.alzforum.org/new/detail.asp?id=1790 ;last accessed 01 July 2011). PK data with this compound shows good BBB penetration and a proof of concept study in man has shown a lowering of plasma Aß. The first gamma secretase inhibitors to be tested clinically also reduced plasma Aß in human volunteers (Siemers et al., 2005), but have not gone on to deliver the promise expected. Phase III trials of the gamma secretase inhibitor semagacestat (LY-450139; Lilly) were halted in 2010 due to a worsening of cognitive state and an increased incidence of skin cancer (http://newsroom.lilly.com/releasedetail.cfm?ReleaseID=499794 ;last accessed 01 July 2011). Semagacestat is not Notch-sparing and the skin cancer may be a product of inhibition of Notch signalling (see Section 2.ii.b); the worsening of clinical state is somewhat more difficult to explain. Flurizan (r-flurbiprofen; Tarenflurbil; Myriad Pharmaceuticals) is a gamma secretase modulator showing specificity for inhibition of gamma secretase over other substrates. Despite showing promise in transgenic mice studies (Eriksen et al., 2003), and in phase II trials, a large phase III study was disappointing and the drug has been discontinued for AD (Imbimbo, 2009). Some positive news from the ongoing clinical trials of the Notch-sparing inhibitor GSI-953 (Martone et al., 2009) is desperately needed, both therapeutically and for support of the amyloid hypothesis.

Subsequent to the beta and gamma processing of APP and the generation of soluble Aß, the key pathogenic feature in AD is proposed to be the aggregation/fibrillization of the amyloid peptide. This essentially physical interaction, initially between Aß monomers to form multimers of increasing size (and presumed neurotoxicity) has attracted enormous interest as an obvious therapeutic intervention point. Despite the discovery of many diverse, seemingly unrelated chemical structures capable of interfering with the aggregation process, only a few compounds have demonstrated efficacy *in vivo* in transgenic mouse models and even fewer have made it to the clinic. Tramiprosate (Alzhemed/Homotaurine; Neurochem) is a glycosaminoglycan (GAG) mimetic and, as such, it inhibits the amyloidogenic interaction between GAGs and Aß. When administered to APP transgenic mice (TgCRND8), the compound inhibited Aß-related pathology (Gervais et al., 2007). Large scale Phase III trials of tramiprosate failed to demonstrate any convincing clinical efficacy, possibly due to a range of non-pharmacologically related problems (http://www.alzforum.org/new/detail.asp?id=1647; last accessed 01 July 2011) although there was some suggestion of a beneficial effect on cognition together with a decrease in hippocampal atrophy in the treatment group (Gauthier et al., 2009, Saumier et al., 2009). ELND005 (AZD-103/ Scyllo-inositol; Elan/Transition Therapeutics) has been shown to inhibit Aß aggregation *in vitro* and *in vivo* (McLaurin et al., 2006). Oral administration of the compound to TgCRND8 mice attenuated the changes in synaptic and Aß pathology evident in these mice (McLaurin et al., 2006). Phase II clinical trials of ELND005 have indicated evidence of serious side effects at the higher doses (1000 mg and 200 mg bid) although Elan and Transition Therapeutics have announced that they are moving to Phase III with a lower dose (250 mg bid) (http://www.news-medical.net/news/20100810/Elan-and-Transition-Therapeutics-report-results-of-ELND005-Phase-2-study-for-Alzheimers-disease.aspx; last accessed 01 July 2011).

An observation from epidemiological studies that the use of NSAIDs was reflected in a reduced risk of AD (McGeer et al., 1996) has led to many *in vitro* and *in vivo* attempts aimed at providing a link between the mode of action of anti-inflammatory drugs and some facet of AD pathogenesis. A series of studies in APP transgenic mice demonstrated that Aß pathology was reduced by the administration of a variety of NSAIDs (Lim et al., 2000; Sung et al., 2004; van Groen and Kadish, 2005; Choi et al., 2010). Unfortunately, clinical trials with NSAIDs have not supported the potential for disease modification in AD where studies have either reported no effect on disease progression (de Jong et al., 2008; Reines et al., 2004) or a worsening of cognitive state (Arvanitakis et al., 2008; Brietner et al., 2009; Sonnen et al., 2010). The reason for this discord from the positive effects observed in transgenics is not clear although there is a suggestion that some beneficial effects may be apparent if apolipoprotein E4 status is taken into consideration (Pasqualetti et al., 2009; Cole and Frautschy, 2010).

A few other compounds that have been reported to reduce Aß pathology in transgenic mice are worthy of mention. The Cu-Zn chelator clioquinol decreased Aß load and plaque deposition in Tg2576 mice (Cherny et al., 2001). The subsequent programme of development within Prana Biotechnology has led to the synthesis of the novel 8-hydroxyquinoline derivative PBT-2. This compound has been reported to improve cognitive scores in AD patients in a Phase IIa study (Faux et al., 2010); further trials are ongoing.

A previous review of APP transgenic mouse pathology concluded that "the extent to which these animals prove to be useful models of AD, where drug interference with the Aß deposition system can be translated into clinical efficacy, is yet to be proven" (Howlett and Richardson 2009). The amyloid cascade has provided a range of therapeutic targets, many of which, described above, have seen success in terms of inhibitors producing decreases in Aß plaque and associated inflammatory pathology. Sadly, this has not

translated into convincing clinical efficacy and has even, in some cases, appeared to lead to a worsening of cognitive status. Does this mean that the pre-clinical transgenic models are not appropriate or that the hypothesis is flawed?

In the case of the former, potential therapeutic agents have usually been shown to be effective in young APP transgenics, during the period of onset of plaque deposition and only in some cases have agents been shown to be effective against ready formed plaque. The plaques in APP transgenic mice, although exhibiting some chemical modification (eg. N-terminal truncation), do not show the pronounced modification and cross-linking found in AD brain (Kuo et al., 2001; Kalback et al., 2002). Furthermore, it is highly likely that plaque load is fairly substantial with neurodegenerative changes taking place before clinical diagnosis is made – and, as commented above, neurodegenerative changes in APP transgenics, even when observed, are not of the magnitude occurring in AD brain. Thus, by the time that clinical intervention begins in man it may already be too late to affect the disease course by simply preventing amyloid deposition or even removing what is already deposited. The lack of neurofibrillary tangles and associated pathology is also a major issue with APP transgenic mice, other than those with the expression of a mutant tau transgene, as in the 3xTg-AD discussed later.

*ii) Moving on – new developments involving the use of transgenic mice.*

a. Vaccination Approaches

The studies described above are the first attempts to take a compound that lowers Aß in transgenic mice into the clinic to AD patients as a putative disease-modifying therapeutic. The clinical trials have highlighted major issues with safety and efficacy; subsequent efforts have been directed towards overcoming these problems. The PDAPP mouse, bearing an APP.Indiana minigene, first described by Games and colleagues in 1995 (Games et al., 1995), provided a means for investigating immunotherapeutic approaches. Thus, immunisation of these mice with aggregated Aß1-42 led to a diminution of plaque pathology in aged PDAPPs with established Aß pathology and an attenuation of the development of pathology in younger animals (Schenk et al., 1999). Subsequent to this report, similar data emerged from other groups using other APP transgenic mice and consistently showed decreases in plaque number, in associated inflammatory responses and, in some cases, a reversal of cognitive impairment (Janus et al., 2000; Morgan et al., 2000). As noted above, clinical trials involving immunisation with Aß peptide had to be halted for safety reasons. Many alternative approaches to active vaccination have been or are being investigated ("active vaccination" meaning immunisation with Aß or an Aß fragment, either alone or complexed with some other agent). For instance, the vaccine CAD106 (Novartis) is composed of multiple copies of Aß1-6 coupled to a Qß virus-like particle; CAD106 has been shown to decrease amyloid accumulation in APP transgenic mouse brain (http://www.alzforum.org/drg/drc/detail.asp?id=133 ;last accessed 01 July 2011). Clinical trials of CAD106 are ongoing (http://clinicaltrials.gov/ct2/show/NCT00795418; last accessed 01 July 2011). Preclinical studies involving immunization with "amplicons" that co-deliver Aß1-42 with herpes simplex virus and interleukin-4 in an attempt to facilitate an appropriate immune response has resulted in reduced amyloid pathology in transgenic mice (Frazer et al., 2008). Similarly, immunization with an adenoviral-linked Aß1-43 decreased plaque pathology and Aß load (Mouri et al., 2007) and an adenoviral-quadrivalent foldable Aß1-15 construct, generated in an attempt to minimise T-cell responses, also resulted in decreases in Aß deposition (Zou et al., 2008) and a single injection of adenoviral cholera toxin B linked to Aß1-42 was able to decrease both Aß plaque and associated astrogliosis (Zhang et al., 2003).

One of the potential issues arising from the AN1792 study was the possibility of an inappropriate adjuvant response. Attempts have therefore been made to avoid such adjuvant-related problems eg. with the use of Aß fragments employing alum adjuvants (Asuni et al., 2006). An alternative approach to facilitating a non-self T helper response has been the use of a chemokine-based DNA epitope vaccine where a fusion protein was expressed consisting of 3 copies of the self-B cell epitope of Aß42 (Aß1-11) , together with a non-self T helper cell epitope (PADRE), and macrophage-derived chemokine (MDC/CCL22) as a molecular adjuvant. This approach successfully reduced Aß plaque load and decreased astrogliosis and microgliosis (Movsesyan et al., 2008). Liposome-coupled Aß1-15 or 1-16 has also been found to reduce amyloid load (Muhs et al., 2007). A further alternative approach has utilised the Aß-binding properties of the 20 amino-acid peptide SDPM1. This peptide was identified as an Aß tetramer binding agent capable of preventing further aggregation (Kang et al., 2003). When administered to APP transgenic mice, SDPM1-induced peptide-mimotope antibodies reduced Aß plaques and Aß load and demonstrated no unwanted T-cell response (Wang et al., 2010). A series of mimotopes (Affitopes) of the N-terminal 1-6 fragment of Aß have been developed by Affiris in the hope of minimising a T-cell response. In preclinical testing in Tg2576 APP.Swe transgenic mice, immunisation with Affitope-derived sera reduced amyloid plaque load by 70% with a parallel decrease in inflammatory cells (Schneeberger et al., 2009). A recent press release from Affiris has claimed promising results from an early clinical study (http://www.affiris.com/html/en/presse_medien/pressemeldungen.html; last accessed 01 July 2011).

An interesting and novel approach involves immunisation with a peptide to produce a

conformational selective immune response. British Dementia is associated with the accumulation of the ABri protein (Vidal et al., 1999), an amyloid protein chemically unrelated to Aß. Immunisation with a polymerised ABri related-protein induced what appears to be a non-specific beta-sheet conformational antibody that reduced Aß pathology in APP.Swe x PS1.M146L mice and also bound to paired helical filaments on AD tissue sections (Goni et al., 2010). Other attempts at improving antibodies have included deglycosylation of the antibody prior to administration, a procedure that, in addition to decreasing parenchymal Aß deposits, also decreased cerebrovascular amyloid and the incidence of microhaemorrhages (Wilcock et al., 2006). Aside from targeting Aß itself, alternative immunotherapeutic approaches include targeting the N-terminal Aß (ß-secretase) cleavage site on APP which reduces Aß pathology in an APP.London mouse (Arbel-Ornath et al., 2010).

In the brain of AD subjects, a significant proportion of plaque Aß is N-terminally truncated and occurs in pyro-3-glutamate (p3NE-Aß) form (Kuo et al., 1997). In APP transgenic brain, such as the TASTPM, a proportion of the Aß also exists in this form (Howlett et al., 2008). Immunisation of 5xFAD mice with an antibody to oligomeric p3NE-Aß reduced both overall plaque pathology and levels of the p3NE-Aß form (Wirths et al., 2009). In AD brain sections, this antibody labelled intraneuronal and cerebrovascular Aß but not extracellular plaques. Furthermore, plasma from AD patients had reduced levels of p3NE-Aß when assayed by an elisa utilising this antibody. Hence the significance of the presence of p3NE-Aß in plaques is not fully understood thus questioning the utility of a p3NE-Aß specific antibody approach.

One note of caution reported concerns the potential for optical complications that have been indentified following vaccination with Aß peptides. Despite reducing retinal Aß deposits, active vaccination increased microvascular Aß and produced local neuroinflammation (Liu et al., 2009). As commented by these researchers, findings in other similar studies where vision is compromised could easily lead to misinterpretation of effects on cognitive deficits.

b. BACE1 and gamma secretase inhibition

By virtue of their tendency towards a peptidergic nature, the poor PK profile of BACE1 inhibitors has rather limited their progress in development although there have been a few reports of progress being made in the discovery of orally active inhibitors of brain BACE1 activity (Hussain, 2010). More recently, a non-peptidergic orally active compound, TAK-070 has been shown to decrease brain Aß and plaque load in Tg2576 (Fukumoto et al., 2010) thus demonstrating the realistic possibility of an orally active BACE1 inhibitor reaching the clinic. An alternative means of regulating BACE1 has been to decrease its expression. Treatment of APP.Swe x PS1.M146L mice chronically by implant with the NFkappaB inhibitor celastrol inhibited BACE1 expression and led to decreases in Aß plaque and microglial activation (Paris et al., 2010).

Of some concern is data suggesting that although BACE1 inhibitors are equally effective against wild-type and Swedish APP cleavage *in vitro*, the two APPs have differing subcellular locations and thus possibly differing spatial relationships exist between APP, BACE1 and BACE1 inhibitors (Yamakawa et al., 2010). Hence, the use of transgenic mice expressing APP.Swe may not be appropriate for anything other than searching for inhibitors of cleavage of that particular mutated APP. In support of this view, two inhibitors of cathepsin B reduce plaque load in APP London mice (that express the wild-type BACE1 site) but not in Tg2576 animals with the Swedish mutation at the cleavage site (Hook et al., 2008).

Efforts to design inhibitors of gamma secretase have been faced with the additional hurdle of sparing Notch cleavage. Gamma secretase cleaves a number of substrates including the transmembrane protein Notch. Inhibition of Notch cleavage has implications for B- and T-cell maturation (Deftos et al., 2000) and for the induction of gastrointestinal toxicity (Searfoss et al., 2003). Notch sparing, at least in APP transgenic mice, has been achieved by the development of so-called gamma-secretase modulators that reduce Aß plaque deposition but avoid inhibition of γ-cleavage of Notch and therefore protect against the toxicity associated with gamma secretase inhibitors arising from decreases in the Notch cleavage products (Kounnas et al., 2010). Other potential issues with gamma secretase inhibition are effects on dendritic spine density and neurogenesis. Although APP (-/-) mice have a greater number of spines than wild-type mice, inhibition of gamma secretase in these animals with the inhibitors DAPT or LY450139 decreased dendritic spines indicating an APP-independent process (Bittner et al., 2009). Furthermore, Tg2576 mice treated chronically with the gamma modulator CHF5074, although showing decreases in cortical plaque and inflammatory pathology, also exhibited a decrease in doublecortin labelling of neuroblasts in the hippocampus suggesting an unwanted effect on neurogenesis (Imbimbo et al., 2010).

c. Aß Aggregation inhibitors

As noted earlier for tramiprosate, obtaining inhibitors of Aß aggregation *in vitro* and seeing that reproduced as reducing plaque pathology in APP transgenics in vivo is achievable although, to date, there have been no convincing claims for efficacy in man. The first "wave" of aggregation inhibitors sought to simply prevent the formation of a fairly poorly defined multimeric product in vivo and to show decreases in plaque density in APP transgenic mice (eg. Nordberg et al., 2002; Permanne et al., 2002). More recently there has been a refining of the target with the belief that

rather than aggregated, fibrillar Aß being the neurotoxic species, attention should be directed towards preventing the formation of small oligomeric forms (Haass and Selkoe, 2007). With the development of antibodies such as A11, apparently specific for the oligomers in which toxicity is thought to lie (Glabe, 2004), it has become possible to identify agents that have differential effects on Aß plaque development and the geneneration of small oligomeric forms (Hamaguchi et al., 2009). This may signal a step forward in the identification of molecules active in transgenic mice with effects that translate into efficacy in the clinic.

The plant extract curcumin deserves a mention as it possesses a range of activities that suggest that it might have disease modifying properties in AD. Curcumin has anti-aggregatory, anti-inflammatory and anti-oxidative properties and has been shown to reduce Aß and associated pathologies in APP.Swe x PS1dE9 mice (Garcia-Alloza et al., 2007). Although its potential to cross the blood brain barrier is limited, curcumin (Longvida, Verdure Biosciences, also available as a herbal dietary supplement) is currently being directed towards a Phase II trial in moderate to severe AD patients (http://clinicaltrials.gov/ct2/show/NCT01001637; last accessed 01 July 2011).

## 3. Advances in the characterisation of pathology of APP transgenics

### *i) Amyloid pathology*

Some of the doubts about the acceptability of mice bearing FAD mutations as models of sporadic AD have been refuted by data generated with the AD11 anti-NGF mouse that exhibits NGF deficiency and develops a full spectrum of AD-like pathology (Capsoni et al., 2002). Thus, in the absence of mutant FAD transgenes, these mice develop plaque and tangle pathology reminiscent of that observed in AD subjects. The AD11 mice also show significant deficits in glutamatergic plasticity in the dentate gyrus (Houeland et al., 2010) reminiscent of deficits observed in a range of APP FAD transgenic mice (Palop et al., 2007). These abnormalities, therefore, may be a consequence of the AD-like pathology rather than a function of any particular model.

Nevertheless, in simple terms, APP transgenic mice (with or without a mutated presenilin-1 transgene) should be considered as being an incomplete model of AD. Obviously, they develop Aß plaques resembling those found in AD brain, they have associated astrocyte/microglial pathology and they have plaque-associated hyperphosphorylated tau. Nevertheless, in the absence of a mutant tau transgene, they do not have neurofibrillary tangles and they do not, by any stretch of the imagination, have cognitive/behavioural deficits approaching those characterising AD. Moreover, although the transgenic mouse plaques "resemble" those found in AD brain, there are subtle differences. As noted previously, diffuse plaques are not an early event in transgenic mouse brain (Howlett and Richardson, 2009) and differences in thioflavin-S labelling of plaques in APP.Swe x PS1.A246E and AD brain also support the notion of differences in amyloid content (Meadowcroft et al., 2009). This latter report also comments on the differences in iron content of plaques in the two species, an observation that may suggest differences in oxidative status. Amyloid deposits in transgenic mouse brain are more easily extractable than those in AD brain which are fairly insoluble, requiring formic acid treatment for their dissolution (Kalback et al., 2002). A recent development in APP transgenic mouse lines has been the incorporation of an E693G 'Arctic' mutation to produce an APP.ArcSwe mouse (Philipson et al., 2009). This mouse is characterised by dense, insoluble plaques, rather like those found in AD brain. Whilst families with the Arctic mutation are very rare, the data does emphasise differences between plaques in AD brain and those in the more common APP.Swe models and a case could probably be made for employing a model of the ArcSwe type in studies directed towards plaque removal. A further potential advance in model development is the recently discovered FAD mutation APP.E693 delta species lacking Glu-22 which is characterised by a lack of Aß plaques (Tomiyama et al., 2008). *In vitro*, the Aß-Glu22 deletion peptide oligomerises but does not produce aggregates. From 8mo of age, E693delta transgenic mice show intraneuronal Aß oligomers, abnormalities in tau phosphorylation and decreases in synaptophysin accompanied by impairments in LTP and memory (Tomiyama et al., 2010). Although further characterisation of these mice is required, the model is supportive of a view that oligomers rather than large aggregated Aß are a pathogenic species.

A significant number of FAD cases are attributable to mutations in the PS1 gene (Lleo et al., 2004). Transgenic mice overexpressing mutant human PS1 alone (ie. no human APP transgene), although not developing Aß deposits, show elevated brain levels of Aß protein (Borchelt et al., 1996; Duff et al., 1996). Furthermore, mice expressing PS1.M146L and PS1.P117L transgenes show vascular changes in the form of a thinning of the microvasculature and a tendency for vessels to form abnormal loops (Gama Sosa et al., 2010). In a more extreme model, the 3xTg-AD mouse has microvessels with thicker basement membranes in the walls than seen in wild-type mice; a decrease in hippocampal vascular volume was also apparent in these animals (Bourasset et al., 2009).

It has been shown that BACE1 is elevated in the vicinity of Aß plaques in post-mortem AD brain, raising the possibility that defects in the expression of the enzyme could be a pathogenic feature of the disease. Observations in APP transgenic mice, however, show that BACE1 protein is elevated in the absence of any change in BACE1 mRNA suggesting that the BACE1 increases are a response to the increases in APP or Aß (Zhao et al., 2007). Although a positive feedback mechanism appears to exist for BACE1 in its response to

increased APP/Aß production/expression, external stimuli can elicit changes in BACE1 protein itself that can regulate APP processing. For example, a functional deprivation arising as a result of unilateral naris occlusion in Tg2576 mice led to increases in BACE1 immunoreactivity and Aß deposition in the ipsilateral olfactory bulb and piriform cortex (Zhang et al., 2010)

The fundamental role played by BACE1 in Aß deposition has been demonstrated in a number of studies. In two APP transgenic mouse lines (APP.Swe x PS1dE9 and 5xFAD), immunoreactivity for BACE1 and Aß was found in swollen presynaptic terminals and fine axonal processes (Zhang et al., 2009). The processes exhibited sprouting and swelling associated with what appeared to be release of Aß into the extracellular parenchyma suggesting that increases in BACE1 and/or Aß within the terminal were responsible for the beginnings of plaque development. Exactly how (or even 'if') this release of Aß leads to degenerative effects remains far from clear. PDAPP x BACE(-/-) mice show that BACE1 is essential for the production of Aß and associated pathology. Interestingly, a PDAPP x BACE(+/-) cross demonstrated only a 12% decrease in Aß load in young animals but a dramatic loss of the pathological features of PDAPPs in older mice (McConlogue et al., 2007). The precise role of BACE1 in the pathogenesis of plaques is not completely clear although a detailed immunohistochemical study suggests a continuing process from a presence in synaptic boutons and fine axonal processes to small clusters of dystrophic neuritis to the earliest plaques and so on with BACE1 being present at every stage (Zhang et al., 2009)

The time-frame for plaque development in AD is not easy to assess; in an APP.Swe x PS1dE9-YFP model, *in vivo* multiphoton microscopy showed that plaques appeared to form over 24h with activated microglia appearing within 1-2 days (Meyer-Luehmann et al., 2008). The relative insolubility of AD amyloid plaques may not, however, simply be a product of their prolonged lifespan during the progression of the disease but rather reflect differences in the conformational status of the amyloid within the plaque.

In AD brain, much of the deposited Aß is N-terminally truncated and often shows pyro-3 glutamate immunoreactivity (Saido et al., 1995; Kuo et al., 1997). Similar pyro-3 glutamate truncated forms of Aß have also been reported in TASTPM and 3xTg-AD transgenic mice and in senile macaques and baboons (Howlett et al., 2008; Hartig et al., 2010). Pyroglutamate forms of Aß may be important in disease pathogenesis since they are resistant to proteolysis, aggregate rapidly and are toxic to neurons (Tekirian et al., 1999). In APP/PS1.KI mice, p3NE-Aß positive plaque load increased with age while the labelling of plaques with an Aß 1-x antibody decreased suggesting an ongoing N-terminal cleavage of deposited Aß (Wirths et al., 2010a). Although such chemical modifications may be viewed as a relatively late event in plaque formation, inhibiting the formation of the p3NE truncated form of Aß with a glutaminyl cyclase inhibitor decreased plaque formation and associated gliosis and improved cognition in Tg2576 mice (Schilling et al., 2008).

### *ii) Inflammatory pathology*

It is widely accepted that inflammation forms a fundamental facet of AD pathology. Inflammatory cells, such as astrocytes and microglia, are found in close contact with Aß deposits in both AD and transgenic mouse brain although the role of the inflammatory process in the disease is far from clear. On one hand, based on the epidemiological evidence from NSAID studies, it might appear that Aß production/deposition initiates a microglial response resulting in the release of cytotoxic molecules and cytokines. Furthermore, the protective effect of NSAIDs, at least in mice (discussed above), might suggest that an inflammatory response promotes Aß deposition. Conversely, it has been suggested that microglia are not essential for deposition or maintenance of either Aß plaques or the associated dystrophic neurites, since removal of microglia from APP23 and APP.Swe x PS1.L166P mice had no effect on the pathology (Grathwohl et al., 2009). Other reports, however, have supported a role for microglia in disease progression with VEGF-1, the receptor for VEGF, being identified immunohistochemically to be associated with microglia in AD brain and to be increased in brain tissue from AD patients and in human microglia stimulated with the peptide (Ryu et al., 2009). VEGF-1 was also increased in hippocampus from rats injected with Aß1-42; treatment of these animals with a VEGF-1 antibody resulted in a reduction in microglial motility and a resultant neuroprotective effect. Furthermore, clearance of Aß (demonstrated for oligomeric forms) may be CD-45 microglia-mediated since a deficiency in CD45 in APPxPS1 mice results in increases in intra- and extra-cellular soluble oligomeric and insoluble aggregated Aß, together with an increased expression of neurotoxic cytokines and neuronal loss (Zhu et al., 2011). Although transgenic mice expressing the P301L mutation show evidence of tangle accumulation and microglial activation, the induction of an inflammatory response arising from the injection of LPS into the hippocampus and frontal cortex accentuated tau phosphorylation (Lee et al., 2010a). In 3xTg-AD mice, eliciting an immune response by LPS injection increased Aß pathology, an effect that could be inhibited by blocking TNF signalling (McAlpine et al., 2009). Conversely, in an earlier study, a similar dosing regimen and inflammatory response was associated with a decrease in Aß (Herber et al., 2007). The overexpression of murine IL-6 in the brains of TgCRND8 mice induced a significance gliosis and up-regulation of glial phagocytotic markers (Chakrabarty et al., 2010). This was accompanied by an attenuation of Aß deposition probably arising as a consequence of microglial mediated phagocytosis of Aß. Furthermore, loss of the CX3CR1 microglial receptor, which is believed to modulate the phagocytosis of protofibrillar,

but not fibrillar Aß (Liu et al., 2010b) and which results in a decrease in neuronal-microglial signalling, resulted in a facilitation of pathology in APP transgenic mice (Lee et al., 2010c).

Support for the phagocytosis of Aß by microglia has also come from a study demonstrating a dependency on presenilin 1 and 2 since presenilin-depleted mice have reduced microglial activity and a deficiency in Aß phagocytosis (Farfara et al., 2011). Microglia appear to be part of the response to Aß antibody vaccination; phagocytosis of deposited Aß by microglia is a possible means of clearance (Bard et al., 2000). Adminstration of m3D6, an antibody that binds both soluble and fibrillar Aß, to older PDAPP mice expressing a CX3/CR1-GFP gene in order to label microglia fluorescently, resulted in a marked increase in microglia and microglial processes while vaccination with a soluble-Aß specific antibody or vaccination of younger PDAPPs did not produce a microglial response (Koenigsknecht-Talboo et al., 2008). Hence the response may be dependent on the Aß species present and the specificity of the antibody.

Conversely, astrocytes may play a role in plaque emergence/maintenance since connexins -30 and -43, which are involved in inter-astrocyte communication, are locally elevated in the vicinity of core plaques in APP x PS1 mice (Mei et al., 2010). S100B is a calcium binding protein expressed by mature astrocytes and generally represents a response to injury. A Tg2576 x human S100B overexpression mouse was characterised by an increased parenchymal and cerebrovasculature Aß and changes in APP cleavage products indicative of an activation of BACE-1 (Mori et al., 2010). These mice also exhibit astrocytosis, microgliosis and an increase in inflammatory cytokines.

What has emerged from studies such as those described above is that the role of the inflammatory process in AD is far from clear. The inflammatory response observed in AD brain may be a disease-promoting process or a protective action. Consequently, it is difficult (and possible dangerous) to consider that interfering with these inflammatory mechanisms is a rationale approach to AD treatment.

### *iii) Mitochondrial dysfunction*

A role for mitochondrial dysfunction in AD pathogenesis has also been considered. In female 3xTg-AD mice, dysfunctioning mitochondria are apparent throughout the life of the animal, from embryo to death, with effects becoming particularly noticeable in the reproductive senescence phase in female mice (Yao et al., 2009; Fattoretti et al., 2010). It is believed that mitochondrial defects result in decreased neurogenesis in adult mice, as assessed by doublecortin labelling of hippocampal subgranular zone neuroblasts (Calingasan et al., 2008). Interestingly, neurogenesis was restored in 3xTg-AD mice by the peripheral administration of an 11-mer peptide based on the active region of ciliary neurotrophic factor (CNTF, amino acid residues 146-156) (Blanchard et al., 2010). Although this treatment enhanced cognition, it did not have any effect on amyloid or tau pathology. The relationship between cognition and tau has also been questioned by a study where the drug minocycline reversed the anti-inflammatory profile in 3xTg-AD mice. Despite reducing insoluble and soluble Aß, GFAP, TNF alpha and IL6 and overturning the cognitive deficits observed in these animals, minocycline had no major effect on phosphotau pathology (Parachikova et al., 2010). These findings are in agreement with earlier studies in 3xTg-AD mice where immunisation with a range of Aß antibodies failed to clear aggregates of hyperphosphorylated tau, despite positive effects of Aß and cognition (Oddo et al., 2004). Thus, phosphotau accumulation as a consequence of a human tau transgene may not be particularly responsive to changes in Aß.

### *iv) Neurodegeneration and changes in dendritic pathology*

A major criticism of APP transgenic mice in general has been that they display limited evidence of neurodegeneration, even in the presence of a heavy amyloid load (Howlett and Richardson, 2009). A comparison between transgenic mouse lines and human AD, preclinical AD and aged non-demented subjects, showed that both Tg2576 and TgCRND8 mice had dystrophic neurites that were morphologically and neurochemically similar to those observed in aged human and preclinical AD brain (Woodhouse et al., 2009). The mouse brains and aged controls/preclinical AD cases were characterised by the lack of hyperphosphorylated-tau dystrophic-neurite pathology although, in each case, there were neurites containing neurofilament triplet protein and alpha-internexin. In contrast, dystrophic neurites from AD cases were typified by hyperphosphorylated tau labelling but no neurofilament triplet protein. It might be argued, therefore, that the mouse models appear to have more in common with an aged control or preclinical AD stage as opposed to the mild to moderate subjects that are the usual targets in clinical trials.

There have also been indications that dendritic spines may be altered in APP transgenic mice. In APP.Swe x PS1dE9 mice, the morphology of dendritic spines associated with plaques was found to be altered and there was a decrease in large spine density in plaque-free regions (Knafo et al., 2009a). Association studies two decades ago led to the discovery that apolipoprotein E4 (ApoE4) is a risk factor for AD (Pericak-Vance et al., 1991). Exactly how that risk is conferred has still not been established but ApoE4 mice show dendritic spine loss from 4 weeks to 1 year of age while ApoE2 mice exhibit enhanced dendritic tree formation (Dumanis et al., 2009). APP.Swe x PS1dE9 mice also exhibit a decrease in the density of large dendritic spines in the amygdala (Knafo et al., 2009b). This is an area essentially with very few Aß plaques and

no neuronal loss. The amygdala is vital for the establishment of cued fear conditioning and in 12-14 month old APP.Swe x PS1dE9 male mice the fear conditioning response was diminished (Knafo et al., 2009b). A recent report has proposed calcineurin activation as a mediator of neurodegeneration with associated dystrophic neurites and loss of dendritic spines (Wu et al., 2010) while other data has pointed to deficiencies in proteasome function or cAMP/PKA/CREB signalling being involved in dendritic insufficiency (Smith et al., 2009).

Although Aß is concentrated in plaques, there are substantial concentrations in non-plaque tissue, as demonstrated by laser-capture microdissection (Howlett et al., 2008), and it may be that soluble, non-deposited Aß is responsible for the dendritic changes. Indeed, synaptic impairment is apparent in Tg2576 mice before plaque deposition occurs pointing to a soluble-Aß driven defect (Hermann et al., 2009). It is also possible that the presence of the PS-1 protein plays a role in the changes in dendritic spines and synaptic function in the APPxPS1 double transgenics since mice overexpressing either the wild-type human PS-1 protein or the L286V mutant alone (ie. no human APP transgene) showed transient synaptic dysfunction (Auffret et al., 2009).

The precise reason for the lack of overt neurodegeneration in APP transgenic mice is not clear. In APP x PS1 mice, an upregulation of the anti-apoptotic protein Bcl-2 was demonstrated to be associated with neuroprotection (Karlnoski et al., 2007) and 3xTg-AD mice overexpressing Bcl-2 show a reduced caspase cleavage of tau and a decrease in neurofibrillary tangles (Rohn et al., 2008). These latter mice also show an attenuation of APP processing, reflected in increases in intracellular APP and decreases in Aß. In AD brain, Bcl-2 increases with disease progression although it has been claimed that the protein is reduced in tangle bearing neurons (Satou et al., 1995). It is tempting to speculate, however, that a loss of the protective effects of Bcl-2 upregulation in AD plays a role in the disease process. There is also evidence of extensive autophagic pathology in AD brain although its role in the disease is not known (Nixon et al., 2000). Enhancing lysosomal cathepsin activity by cystatin B deletion in TgCRND8 mice reduced most of the autophagic, lysosomal and amyloid pathology and prevented cognitive deficits (Yang et al., 2011). Somewhat conversely, although not an APP mouse study, the administration of the disaccharide trehalose (which enhances autophagic activity) to mice deficient in parkin but overexpressing tau (a tauopathy, Parkinson's disease model), produced an upregulation of autophagy markers and an accompaning decrease in phosphorylated tau and associated astrogliosis (Rodriguez-Navarro et al., 2010) suggesting a protective effect.

The lack of obvious neurodegeneration (and tangle pathology) in many APP transgenic models remains a major concern given the nature and extent of the neuronal loss occurring in the human condition. Nevertheless, careful analysis of APP mouse brain does reveal evidence of neurodegeneration. In the APP.Swe x PS1dE9 mouse, the developing Aß pathology is accompanied by axonal degeneration and a loss of monoaminergic neurons (Liu et al., 2008b). A more "extreme" model is the APP/PS1KI mouse (APP.Swe + London with murine Thy-1 promoter; PS1.M233T + L235P under control of endogenous mouse PS1 promoter) that shows extensive loss of CA1 neurons and severe axonal degeneration (Casas et al., 2004; Wirths et al., 2007) including inflammatory changes in both the brain and spinal cord (Wirths et al., 2010b). Thus, although showing promise with respect to neurodegenerative changes in the CA1, the latter pathology is not particularly representative of AD and data from such a mouse model requires careful interrogation.

### *v) Other Pathologies*

Reelin is a protein that controls neuronal positioning during brain development. Decreased reelin expression is found in the brains of APP transgenic mice and AD subjects (Chin et al., 2007). Reelin signalling is associated with both tau phosphorylation and amyloid processing while dysfunctional signalling leads to defects in brain development and hyperphosphorylation of tau (Hiesberger et al., 1999). When reelin expression was reduced by crossing an APP transgenic mouse (APP.Swe + E693G) with a heterozygous reeler mouse, increases in plaque size and number were observed compared with the APP transgenic with normal reelin (Kocherhans et al., 2010). In addition, in older mice, silver-stained neurofibrillary tangles were present suggesting that either the deficient reelin signalling had a direct effect on tau phosphorylation or it was mediated via an increase in amyloidogenic APP processing.

The accumulation of α-synuclein in intracellular inclusions is a characteristic of Lewy-body disease. Most cases of sporadic and familial AD, however, also develop Lewy-body like inclusions (Hamilton, 2000). Double transgenic mice overexpressing APP and human α-synuclein show an increase in the accumulation of α-synuclein, enhanced cognitive impairment and neurodegeneration although the mice were reported to exhibit little change in plaque deposition (Masliah et al., 2001). Introducing a human α-synuclein transgene into 3xTg-AD mice produces an increase in plaque and tau pathology (compared to 3xTg-AD alone) and α-synuclein pathology (Clinton et al., 2010). The lack of change in APP or C-terminal metabolites in the 3xTg-AD + α-synuclein mice reported by Clinton and colleagues may point to a purely synergistic aggregatory effect between proteins susceptible to ß-sheet formation. The possible cross-talk observed between Aß and α-synuclein is also apparent between Aß and prion proteins. Tg2576 mice inoculated with prions showed an

acceleration of both amyloid and prion pathology (Morales et al., 2010) again indicating a synergism between misfolded proteins.

Protein folding is part of the normal process that converts newly synthesised proteins to physiologically functional molecules. Many proteins are handled through the secretory pathway where they are transported to the lumen of the endoplasmic reticulum (ER) for folding to occur. Misfolding can arise as a result of genetic mutations, mistranslation, environmental factors, change in redox state/oxidative stress and so on. Cellular proteins that fold incorrectly have the potential to induce cellular damage. These proteins can be targeted for degradation by the unfolded protein response (UPR). The accumulation of misfolded proteins within the endoplasmic reticulum (ER) results in a specific UPR that, when activated, can lead to reduced ER stress or to apoptotic cell death (Mori, 2000). However, while a series of UPR-related genes and proteins were shown to be increased in AD brain, there was no evidence of a similar induction in Tg2576 brain tissue suggesting an important pathological difference between the transgene-derived condition and the human disease (Lee et al., 2010b).

The protein clusterin (apolipoprotein J) has been shown to be associated with rapid clinical progression in AD. The demonstration of elevated plasma clusterin correlating with fibrillar Aß in the medial temporal lobe of AD patients gives rise to the consideration of clusterin as a possible biomarker for the disease (Thambisetty et al., 2010). The precise relationship between brain clusterin, brain Aß and plasma clusterin/Aß is not clear. PDAPP x clusterin -/- mice have fewer Aß plaques and show a reduction in neuritic dystrophy (DeMattos et al., 2002). In the brains of TASTPM mice, endogenous clusterin is found in association with both Aß40 and 42 and there is a fairly robust temporal relationship between clusterin and both forms of the amyloid beta-protein in senile plaque-like deposits (Thambisetty et al., 2010; Howlett, unpublished). Clusterin is cleared from the brain partly through the low density lipoprotein receptor related protein (LRP-1) and it has been suggested that this system may favour the clearance of certain forms of Aß-clusterin complexes (Deane et al., 2009). In fact, Aß peptides with an internal mutation (eg. Dutch, Iowa mutations that result in very pronounced cerebrovascular angiopathies) have reduced binding affinity for LRP-1 resulting in less effective clearance and accumulation in the vessel wall. It is known that LRP1 also plays a crucial role in the maintenance of synaptic/neuronal integrity by virtue of its role in regulating brain lipid metabolism; the brains of transgenic mice deficient in LRP1 exhibit many pathological characteristics of ageing, including neuronal cell loss (Liu et al., 2010a). Further studies are necessary before it can be judged whether the Aß/clusterin/LRP-1 system in transgenic mice bears any resemblance to that in man and, particularly, in AD brain.

## 4. New approaches to the modulation of amyloid-related pathology

It has been known for many years that the stimulation of muscarinic M1 and M3 receptors increases the secretion of sAPPα, driving APP processing away from the amyloidogenic route (Nitsch et al., 1992). Conversely, APP transgenic mice lacking M1 receptors (J20 x M1.KO) have decreased release of sAPPα and an increase in Aß plaque pathology (Davis et al., 2010) supporting the notion of M1 stimulation as a therapeutic target for AD. Similarly, a Tg2576 x nicotinic α-7 acetylcholine receptor (α-7nAChR) knock-out mouse was characterised by an increase in Aß and in severity of deficits in learning and memory (Hernandez et al., 2010). In PDAPP mice, however, knockout of α-7nAChRs, protected against loss of synaptophysin and MAP2 immunoreactivity, reduced gliosis in the mice, but without any changes in APP or Aß (Dziewczapolski et al., 2009). Additionally, the knockout also protected the PDAPP against loss of LTP and defects in cognition. The reason for this disparity is not clear but may reside in the different mutations used. It has been suggested that the Aß 12-28 sequence is important for binding to α-7nAChR (in which case why would a Swedish mutation mouse differ from an Indiana mutation?) (Wang et al., 2000) although other workers dispute the binding between Aß and the receptor (Small et al., 2007).

Despite the lack of success of anti-inflammatory drugs in preventing AD progression, studies have continued in APP transgenics in attempts to throw some light on the reason why such compounds reduce Aß in rodent models but are not effective in man. Cuello et al., (2010) have reported that in a APP.Swe + Indiana mouse, the anti-inflammatory agent minocycline reduced small intracellular pre-plaque oligomers of Aß and that this was accompanied by a lowering of inflammatory markers and an attenuation of behavioural deficits. A specific targeting of the early stages of plaque development may, therefore, be worth investigating clinically.

Although aimed at tau phosphorylation, a comment on the possible use of lithium is of interest. Lithium carbonate has been in use for a number of decades to treat the mania associated with bipolar disorder although its mechanism of action is not fully understood. It has also been reported to show some promise in amyotrophic lateral sclerosis (Fornai et al., 2008). In tau transgenic mice, treatment with lithium carbonate by gavage decreased aggregated tau in the brain and spinal cord, probably by virtue of its inhibitory effects on glycogen synthase kinase-3, although motor and memory deficits were unaffected (Leroy et al., 2010). Due to the high blood concentration of lithium required to produce this effect it is probably unlikely that lithium carbonate will be considered as a potential therapeutic agent suitable for chronic dosing and for a disease of the elderly, such

as AD. Furthermore, a small clinical trial in AD patients has not provided any encouragement for the idea of lithium being of benefit in AD (Hampel et al., 2009).

As we have seen, the perceived pathogenic nature of Aß together with it being deposited extracellularly has led to Aß being targeted in vaccination studies. Hyperphosphorylated tau, by virtue of its intracellular location, may not, at first site, appear to offer the same "targetability". It has been demonstrated, however, that neuronal uptake of antibodies is not a major issue and may be accelerated in various pathological disorders (Sigurdsson, 2008). Moreover, immunisation of a human tau/PS1 transgenic mouse exhibiting cognitive deficits and accelerated tangle development with a 30-mer tau construct, resulted in clearance of tau and reversal of the impaired cognitive defects (Boutajangout et al., 2010). Tau immunotherapy may, therefore, offer an alternative possibility for a therapeutic agent and will no doubt be the topic of further investigation.

A Swedish population-based study has pointed to an association between the gene COL25A1 and risk of AD (Forsell et al., 2010). COL25A1 encodes the protein collagenous Alzheimer amyloid plaque component (CLAC) that binds to Aß and promotes fibril elongation *in vitro* (Kakuyama et al., 2005). Interestingly, the N-terminus of CLAC found deposited in AD brain is pyroglutamate truncated (Hashimoto et al., 2002). Transgenic mice overexpressing COL25A1 show intracellular Aß accumulation and some evidence of extracellular plaque-like deposits (Tong et al., 2010). This somewhat unusual model demonstrating aggregation and deposition of endogenous murine Aß warrants further investigation along with consideration of the COL25A1/CLAC system as a potential drug target.

## 5. Progress in model development

As a means of inducing protein expression, adeno-associated virus (AAV) vectors have been increasingly used to promote or prevent pathologies associated with AD. The role of the cytokine interferon-gamma in the disease is unclear but 3xTg-AD mice receiving chronic interferon-gamma expression by way of AAV delivery responded by developing enhanced Aß-related pathology and increased neurogenesis but decreased phosphotau pathology (Mastrangelo et al., 2009). This dichotomy requires further exploration but highlights the issues that can be apparent in complex multigenic models.

The cause and effect relationship between plaques and tangles, as depicted in the Amyloid Hypothesis is far from clear although evidence does suggest that Aß production or deposition is the trigger for initiating hyperphosphorylation of tau (Hardy and Selkoe, 2002). The characteristic dual pathology of senile plaques and neurofibrillary tangles found in AD but not found in human mutant APP transgenic mice (with or without a human mutant PS1 transgene), led to the development of mice with human mutant APP, PS1 and tau proteins. Probably the most widely reported of such models is the 3xTg-AD, first described by Oddo and colleagues in 2003 (Oddo et al., 2003). This mouse expresses APP.Swe + PS1.M146V + Tau.P301L and is characterised by age-dependent increases in Aß production and tau phosphorylation leading to plaque deposition and neurofibrillary tangle formation together with synaptic dysfunction. Not all of its pathology, however, is characteristic of AD with both Aß and phosphorylated tau positive neurons being found in the brain stem (Overk et al., 2009). Other interesting features include a decrease in neurogenesis that precedes both Aß plaque deposition and neurofibrillary tangle formation (Hamilton et al., 2010) and, at time points preceding the appearance of amyloid and phosphotau pathology, 3xTg-AD mice show changes in myelin sheath structure and in the expression of oligodendrocyte markers (Desai et al., 2004). There is some evidence that apoptosis may play a role in the development of pathology in 3xTg-AD mice since the additional expression of the anti-apoptotic protein Bcl-2 limited both plaque and tangle formation and improved memory (Rohn et al., 2008). The presence of an apoptotic mechanism in AD, however, is not fully established or understood (Raina et al., 2001, 2003; Vermes et al., 2004). Numerous other models involving the expression of a variety of tau transgenes have been reported. The pathological phenotype of many of these has recently been reviewed elsewhere (Noble et al., 2010).

Several lines of APP transgenic mice show evidence of phosphorylated tau accumulation in dystrophic neurites associated with Aß plaques (eg. Sturchler Pierrat et al., 1997; Moechars et al., 1999). APP.SweDI mice (APPswe + E693Q + D694N), however, possess Aß pathology but show no evidence of tau phosphorylation and no neurodegeneration (Wilcock et al., 2008). Crossing these mice with a NOS2.KO animal, however, induced extensive murine phosphotau pathology with accumulations in perivascular neurons and activated microglia and hippocampal neuron loss despite Aß levels being unaltered (Wilcock et al., 2008; Van Nostrand et al., 2010). Thus, the loss of NOS2 and its protein product iNOS may remove a factor(s) that controls the link between Aß and tau phosphorylation. Furthermore, the accumulation of endogenous mouse phosphotau in these animals appears to be responsible for a neuronal loss that is not evident in mice where phosphorylated tau accumulation is brought about by a mutated human tau transgene (Morrissette et al., 2009).

Transgenic mice dominate the AD models scene although there have been attempts to use other species and which are worthy of a mention. A number of groups have directed efforts towards the development of transgenic rats. A double homozygote rat, the progeny of an APP.Swe x APP.Swe + London cross, has been reported to show Aß deposition by 17-18 months of age (Flood et al., 2009). This group have also reported that, as with APP transgenic mouse models, the time to first deposition could be greatly shortened by the

incorporation of a PS1.M146V transgene, with the parallel induction of gliosis and tau phosphorylation. Rats of this line also showed impaired LTP and cognitive deficits (Liu et al., 2008a). A rat model expressing APP.Swe + Indiana exhibited intraneuronal Aß throughout the cortex and hippocampus from a few weeks of age and age-related extracellular plaque formation from 6 months of age (Leon et al., 2010). Accompanying the development of Aß load, but preceding the appearance of plaques was cognitive impairment, the occurrence of which appeared to parallel the detection of small soluble Aß oligomers.

Amongst non-transgenic models, naturally occurring Aß deposition has been observed in a number of species. Beagle dogs display age-related changes in brain and CSF Aß species comparable to that witnessed in AD subjects (Head et al., 2010). Other species that are being investigated as potential models include zebra-fish (Paquet et al., 2010), drosophila (Cowan et al., 2010), *C. elegans* (Virata and Zeller, 2010)) and non-human primates (Lemere et al., 2004, 2008), to name but a few. Obviously, while some may appear more relevant to the human disease than others, ethical considerations must be taken on board and small, invertebrate species offer many advantages.

## 6. Risk factors and promotion of AD in transgenic mice

There is a close association between AD and diabetes although it is not entirely clear whether diabetes is actually a risk factor (Leibson et al., 1997; Akomolafe et al., 2006). Administering streptozotocin to 3xTg-AD mice induces an insulin-deficient diabetes resulting in an increased plaque pathology, tau phosphorylation and GSK3b activity (Jolivalt et al., 2010), strengthening the evidence for a link between the two conditions.

Understanding the role of hormonal changes in the pathogenesis of AD may help throw some light on the higher incidence of AD in females (Andersen et al., 1999) where it is believed that decreasing post-menopausal hormone levels are a critical factor (Henderson, 2008). Similarly, in males, one age-related change that appears to be linked to the development of AD is the depletion of testosterone (Rosario and Pike, 2008). In 3xTg-AD mice, androgen depletion (by gonadectomy) enhances Aß accumulation in the subiculum, hippocampus and amygdala, an effect that could be prevented by testosterone administration and, to a lesser extent, by estrogen 17-estradiol (Rosario et al., 2006, 2010). Interestingly, tau hyperphosphorylation was only slightly increased by gonadectomy although testosterone treatment reduced this to lower than sham values. Nevertheless, sex hormones appear to play a crucial role in disease progression in AD.

It is recognised from epidemiological studies that high cholesterol is a risk factor for AD. Cholesterol is a major component of senile plaques (Mori et al., 2001), its retention in AD brain is linked to increased BACE1 and gamma secretase activities (Xiong et al., 2008) and it is involved in the generation and clearance of APP cleavage products (Puglielli et al., 2003; Wolozin, 2004). Consequently, interfering with cholesterol metabolism can be viewed as a therapeutic target for AD. Inhibition of cholesterol ester synthesis with an ACAT inhibitor (CP-113-818) or a 3xTg-AD mouse with ACAT.KO reduces amyloid pathology (Hutter-Paier et al., 2004; Bryleva et al., 2010). A further study with the ACAT inhibitor CI-1011, a compound suitable for clinical use, has demonstrated a selective removal of diffuse Aß pathology, decreased astrogliosis and enhanced microglial activation in an APP/Swe.Lon mouse and may warrant further investigation (Huttunen et al., 2010). Cholesterol itself has low permeability at the blood-brain-barrier and is dependent on conversion to 24S-hydroxycholesterol for clearance and subsequent degradation by the liver. Accelerating the conversion of cholesterol to 24S-hydroxycholesterol through the injection of an adeno-associated virus linked CYP46A1 gene responsible for encoding neuronal cholesterol 24-hydroxylase in the cortex and hippocampus of APP23 and APP/PS1 mice reduced plaque and oligomer pathology and reversed cognitive deficits (Hudry et al., 2010).

Cholesterol transport from tissues to liver is mediated, in part, by the HDL receptor, scavenger receptor class B type I (SR-BI). SR-BI also mediates the adhesion of microglia to A, plaques in AD brain where it has been found on astrocytes and vascular smooth muscle cells. Partial deletion of SR-BI in J20 transgenic mice resulted in an increase in plaque pathology and in cerebral amyloid angiopathy (Thanopoulou et al., 2010). In close association with the Aß deposits were SR-BI positive perivascular macrophages suggesting a role for SR-BI in AD and amyloid angiopathy.

Docosahexaenoic acid (DHA) has emerged from epidemiological studies and clinical trials as possessing the ability to reduce the risk of AD. Feeding female APPSwe.PS1dE9 mice with a DHA-enriched diet decreased Aß plaque load (Perez et al., 2010). 3xTg-AD transgenic mice, fed a diet high in saturated and omega-6 fat showed an increase in active JNK and phosphorylated IRS-1 and tau (Ma et al., 2009) thus mirroring changes seen in AD brain. Chronic treatment of these mice with fish oil or curcumin reduced phosphorylated JNK, IRS-1, and tau and prevented the degradation of total IRS-1. Curcumin is generally thought unlikely to be of therapeutic value due to its low blood brain barrier penetrance and potential for toxicity (Mancuso et al., 2011). Other epidemiological studies have shown homocysteine to be a risk factor for development of AD. This state can be reproduced *in vivo*; Tg2576 mice fed a diet deficient in folate and vitamins B6 and B12 show an increase in homocysteine levels and an increase in cortical and hippocampal Aß pathology (Zhuo and Pratico, 2010).

Amongst the non-pharmacological means of reducing plaque pathology, the case for exercise has

gained considerable support. Tg2576 mice subjected to 16 weeks of voluntary or forced running showed fewer thioflavin S positive plaques than sedentary mice. The animals that exercised also had larger hippocampal volumes and attenuated memory impairment (Yuede et al., 2009). Similarly, providing mice with environmental enrichment offers protection against the pathological features characteristic of APP transgenic mice. APPswe.PS1dE9 mice maintained in such an environment display a significant reduction in Aß and hyperphosphorylated tau together with enhanced neurogenesis, axonal transport and long-term potentiation (Hu et al., 2010). Conversely, the stress exhibited by Tg2576 mice restrained daily for 2 hours for 16 days resulted in pathological changes characteristic of AD eg. an increase in plaque deposition, tau hyperphosphorylation and neuritic atrophy of cortical neurons (Lee et al., 2009). Associated with the response was evidence of metabolic oxidative stress which may be a key mediator in disease pathogenesis.

Cerebral hypoperfusion is recognised as a major physiological risk factor for AD (de la Torre, 2010). Bilateral carotid artery occlusion in APP.Swe + Indiana mice produced intense intraneuronal labelling with some evidence of perinuclear staining using an Aß42 specific antibody (Kitaguchi et al., 2009). The precise mechanism behind this localisation is not known but might be expected to reflect an up-regulation of BACE1 activity (Guglielmotto et al., 2009; Zhiyou et al., 2009).

Cerebral amyloid angiopathy (CAA) is a pathology found in most AD cases with deposits of Aß in the vessel walls, similar to those found in parenchymal plaques. The pathogenic consequences of CAA in AD are not understood but it is possible that such vascular changes are linked to haemorrhage or infarction. The change in vessel pathology is related to the degree of CAA when it comes to consideration of associated astrocytes, microglia and pericytes. Wilcock et al., (2009) investigated the pathology of vessels in four mouse models of AD with varying degrees of CAA and found astrocytic changes, particularly in astrocytic end feet. Immunohistochemical data shows that AD pathology (Aß and inflammatory cell responses) is enhanced by endothelin-1 induced lacunar infarcts in APP23 mice suggesting that an inflammatory response may be fundamental in the initiation of AD pathology (Whitehead et al., 2010)

## 7. Conclusions

It is without doubt that the advent of transgenic mouse models of AD has provided a means of approaching therapeutic intervention points that could not be accomplished otherwise. Huge advances have been made in understanding amyloid pathology and its associated features, albeit in an animal model dependent upon the overexpression of 1, 2, 3 or more mutated human transgenes. It is easy to be critical but the increasing magnitude of the AD problem and its cost to society necessitates taking advantage of whatever leads there may be and in the absence of any alternative, unless proven to be completely misleading, APP transgenic mice will continue to be at the forefront of AD research and drug development.

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## References

Akomolafe A., Beiser A., Meigs J.B., Au R., Green R.C., Farrer L.A., Wolf P.A. and Seshadri S. (2006). Diabetes mellitus and risk of developing Alzheimer disease: results from the Framingham Study. Arch. Neurol. 63, 1551-1555.

Andersen K., Launer L.J., Dewey M.E., Letenneur L., Ott A., Copeland J.R., Dartigues J.F., Kragh-Sorensen P., Baldereschi M., Brayne C., Lobo A., Martinez-Lage J.M., Stijnen T. and Hofman A. (1999). Gender differences in the incidence of AD and vascular dementia: The EURODEM Studies. EURODEM Incidence Research Group. Neurology 53, 1992-1997.

Arbel-Ornath M., Becker M., Rabinovich-Toidman P., Gartner M. and Solomon B. (2010). Immunomodulation of APP processing alleviates amyloid--related pathology in Alzheimer's disease transgenic mice. J. Alzheimers Dis. 22, 469-482.

Arvanitakis Z., Schneider J.A., Wilson R.S., Bienias J.L., Kelly J.F., Evans D.A. and Bennett D.A. (2008). Statins, incident Alzheimer disease, change in cognitive function, and neuropathology. Neurology 70, 1795-1802.

Asuni A.A., Boutajangout A., Scholtzova H., Knudsen E., Li Y.S., Quartermain D., Frangione B., Wisniewski T. and Sigurdsson E.M. (2006). Vaccination of Alzheimer's model mice with A beta derivative in alum adjuvant reduces A beta burden without microhemorrhages. Eur. J. Neurosci. 24, 2530-2542.

Auffret A., Gautheron V., Repici M., Kraftsik R., Mount H.T., Mariani J. and Rovira C. (2009). Age-dependent impairment of spine morphology and synaptic plasticity in hippocampal CA1 neurons of a presenilin 1 transgenic mouse model of Alzheimer's disease. J. Neurosci. 29, 10144-10152.

Bard F., Cannon C., Barbour R., Burke R.L., Games D., Grajeda H., Guido T., Hu K., Huang J., Johnson-Wood K., Khan K., Kholodenko D., Lee M., Lieberburg I., Motter R., Nguyen M., Soriano F., Vasquez N., Weiss K., Welch B., Seubert P., Schenk D. and Yednock T. (2000). Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. Nat. Med. 6, 916-919.

Bittner T., Fuhrmann M., Burgold S., Jung C.K.E., Volbracht C., Steiner H., Mitteregger G., Kretzschmar H.A., Haass C. and Herms J. (2009). Gamma-secretase inhibition reduces spine density in vivo via an amyloid precursor protein-dependent pathway. J. Neurosci. 29, 10405-10409.

Blanchard J., Wanka L., Tung Y.C., Cardenas-Aguayo M.C., Laferla F.M., Iqbal K. and Grundke-Iqbal I. (2010). Pharmacologic reversal of neurogenic and neuroplastic abnormalities and cognitive impairments without affecting A, and tau pathologies in 3xTg-AD mice. Acta Neuropathol. 120, 605-621.

Borchelt D.R., Thinakaran G., Eckman C.B., Lee M.K., Davenport F.,

Ratovitsky T., Prada C.M., Kim G., Seekins S., Yager D., Slunt H.H., Wang R., Seeger M., Levey A.I., Gandy S.E., Copeland N.G., Jenkins N.A., Price D.L., Younkin S.G. and Sisodia S.S. (1996). Familial Alzheimer's disease-linked presenilin 1 variants elevate Aß1-42/1-40 ratio *in vitro* and *in vivo*. Neuron 17, 1005-1013.

Bourasset F., Melissa O., Tremblay C., Julien C., Do T.M., Oddo S., LaFerla F. and Calon F. (2009). Reduction of the cerebrovascular volume in a transgenic mouse model of Alzheimer's disease. Neuropharmacology 56, 808-813.

Boutajangout A., Quartermain D. and Sigurdsson E.M. (2010). Immunotherapy targeting pathological tau prevents cognitive decline in a new tangle mouse model. J. Neurosci. 30, 16559-16566.

Breitner J.C.S., Haneuse S.J.P.A., Walker R., Dublin S., Crane P.K., Gray S.L. and Larson E.B. (2009). Risk of dementia and AD with prior exposure to NSAIDs in an elderly community-based cohort. Neurology 72, 1899-905.

Bryleva E.Y., Rogers M.A., Chang C.C.Y., Buen F., Harris B.T., Rousselet E., Seidah N.G., Oddo S., LaFerla F.M., Spencer T.A., Hickey W.F. and Chang T.Y. (2010). ACAT1 gene ablation increases 24(S)-hydroxycholesterol content in the brain and ameliorates amyloid pathology in mice with AD. Proc. Natl. Acad. Sci. USA 107, 3081-3086.

Calingasan N.Y., Ho D.J., Wille E.J., Campagna M.V., Ruan J., Dumont M., Yang L., Shi Q., Gibson G.E. and Beal M.F. (2008). Influence of mitochondrial enzyme deficiency on adult neurogenesis in mouse models of neurodegenerative diseases. Neuroscience 153, 986-996.

Capsoni S., Giannotta S. and Cattaneo A. (2002). Beta-amyloid plaques in a model for sporadic Alzheimer's disease based on transgenic anti-nerve growth factor antibodies. Mol. Cell. Neurosci. 21, 15-28.

Casas C., Sergeant N., Itier J.M., Blanchard V., Wirths O., van der Kolk N., Vingtdeux V., van de Steeg E., Ret G., Canton T., Drobecq H., Clark A., Bonici B., Delacourte A., Benavides J., Schmitz C., Tremp G., Bayer T.A., Benoit P. and Pradier L. (2004). Massive CA1/2 neuronal loss with intraneuronal and N-interminal truncated A beta(42) accumulation in a novel Alzheimer transgenic model. Am. J. Pathol. 165, 1289-1300.

Chakrabarty P., Jansen-West K., Beccard A., Ceballos-Diaz C., Levites Y., Verbeeck C., Zubair A.C., Dickson D., Golde T.E. and Das P. (2010). Massive gliosis induced by interleukin-6 suppresses Aß deposition in vivo: evidence against inflammation as a driving force for amyloid deposition. FASEB J. 24, 548-559.

Chartier-Harlin M.C., Crawford F., Houlden H., Warren A., Hughes D., Fidani L., Goate A., Rossor M., Roques P. and Hardy J. (1991). Early-onset Alzheimer's disease caused by mutations at codon 717 of the beta-amyloid precursor protein gene. Nature 353, 844-846.

Cherny R.A., Atwood C.S., Xilinas M.E., Gray D.N., Jones W.D., McLean C.A., Barnham K.J., Volitakis I., Fraser F.W., Kim Y.S., Huang X.D., Goldstein L.E., Moir R.D., Lim J.T., Beyreuther K., Zheng H., Tanzi R.E., Masters C.L. and Bush A.I. (2001). Treatment with a copper-zinc chelator markedly and rapidly inhibits beta-amyloid accumulation in Alzheimer's disease transgenic mice. Neuron 30, 665-676.

Chin J., Massaro C.M., Palop J.J., Thwin M.T., Yu G.Q., Bien-Ly N., Bender A. and Mucke L. (2007). Reelin depletion in the entorhinal cortex of human amyloid precursor protein transgenic mice and humans with Alzheimer's disease. J. Neurosci. 27, 2727-2733.

Chishti M.A., Yang D.S., Janus C., Phinney A.L., Horne P., Pearson J., Strome R., Zuker N., Loukides J., French J., Turner S., Lozza G., Grilli M., Kunicki S., Morissette C., Paquette J., Gervais F., Bergeron C., Fraser P.E., Carlson G.A., George-Hyslop P. and Westaway D. (2001). Early-onset amyloid deposition and cognitive deficits in transgenic mice expressing a double mutant form of amyloid precursor protein 695. J. Biol. Chem. 276, 21562-21570.

Choi J.K., Jenkins B.G., Carreras I., Kaymakcalan S., Cormier K., Kowall N.W. and Dedeoglu A. (2010). Anti-inflammatory treatment in AD mice protects against neuronal pathology. Exp. Neurol. 223, 377-384.

Clinton L.K., Blurton-Jones M., Myczek K., Trojanowski J.Q. and LaFerla F.M. (2010). Synergistic Interactions between Aß, tau, and alpha-synuclein: acceleration of neuropathology and cognitive decline. J. Neurosci. 30, 7281-7289.

Cole G.M. and Frautschy S.A. (2010). Mechanisms of action of non-steroidal anti-inflammatory drugs for the prevention of Alzheimer's disease. CNS Neurol. Disord. Drug Targets 9, 140-8.

Cowan C.M., Shepherd D. and Mudher A. (2010). Insights from Drosophila models of Alzheimer's disease. Biochem. Soc. Trans. 38, 988-992.

Cuello A.C., Ferretti M.T., Leon W.C., Iulita M.F., Melis T., Ducatenzeiler A., Bruno M.A. and Canneva F. (2010). Early-stage inflammation and experimental therapy in transgenic models of the Alzheimer-like amyloid pathology. Neurodeg. Dis. 7, 96-98.

Davis A.A., Fritz J.J., Wess J., Lah J.J. and Levey A.I. (2010). Deletion of M1 muscarinic acetylcholine receptors increases amyloid pathology in vitro and in vivo. J. Neurosci. 30, 4190-4196.

de Jong E.K., de Haas A.H., Brouwer N., van Weering H.R., Hensens M., Bechmann I., Pratley P., Wesseling E., Boddeke H.W. and Biber K. (2008). Expression of CXCL4 in microglia in vitro and in vivo and its possible signaling through CXCR3. J. Neurochem. 105, 1726-1736.

de la Torre J.C. (2010). Vascular risk factor detection and control may prevent Alzheimer's disease. Ageing Res. Rev. 9, 218-225.

De Strooper B., Annaert W., Cupers P., Saftig P., Craessaerts K., Mumm J.S., Schroeter E.H., Schrijvers V., Wolfe M.S., Ray W.J., Goate A. and Kopan R. (1999). A presenilin-1-dependent gamma-secretase-like protease mediates release of Notch intracellular domain. Nature 398, 518-522.

Deane R., Bell R.D., Sagare A. and Zlokovic B.V. (2009). Clearance of amyloid-beta peptide across the blood-brain barrier: implication for therapies in Alzheimer's disease. CNS Neurol. Disord. Drug Targets 8, 16-30.

Deftos M.L., Huang E., Ojala E.W., Forbush K.A. and Bevan M.J. (2000). Notch1 signaling promotes the maturation of CD4 and CD8 SP thymocytes. Immunity 13, 73-84.

DeMattos R.B., Bales K.R., Cummins D.J., Dodart J.C., Paul S.M. and Holtzman D.M. (2001). Peripheral anti-A beta antibody alters CNS and plasma A beta clearance and decreases brain A beta burden in a mouse model of Alzheimer's disease. Proc. Natl. Acad. Sci. USA 98, 8850-8855.

DeMattos R.B., O'Dell M.A., Parsadanian M., Taylor J.W., Harmony J.A.K., Bales K.R., Paul S.M., Aronow B.J. and Holtzman D.M. (2002). Clusterin promotes amyloid plaque formation and is critical for neuritic toxicity in a mouse model of Alzheimer's disease. Proc. Natl. Acad. Sci. USA 99, 10843-10848.

Desai M.Y., Lima J.A. and Bluemke D.A. (2004). Cardiovascular magnetic resonance imaging: current applications and future directions. Methods Enzymol. 386, 122-148.

Dodart J.C., Bales K.R., Gannon K.S., Greene S.J., DeMattos R.B., Mathis C., DeLong C.A., Wu S., Wu X., Holtzman D.M. and Paul

S.M. (2002). Immunization reverses memory deficits without reducing brain A beta burden in Alzheimer's disease model. Nat. Neurosci. 5, 452-457.

Duff K., Eckman C., Zehr C., Yu X., Prada C.M., Pereztur J., Hutton M., Buee L., Harigaya Y., Yager D., Morgan D., Gordon M.N., Holcomb L., Refolo L., Zenk B., Hardy J. and Younkin S. (1996). Increased amyloid-beta-42(43) in brains of mice expressing mutant presenilin-1. Nature 383, 710-713.

Dumanis S.B., Tesoriero J.A., Babus L.W., Nguyen M.T., Trotter J.H., Ladu M.J., Weeber E.J., Turner R.S., Xu B., Rebeck G.W. and Hoe H.S. (2009). ApoE4 decreases spine density and dendritic complexity in cortical neurons *in vivo*. J. Neurosci. 29, 15317-15322.

Dziewczapolski G., Glogowski C.M., Masliah E. and Heinemann S.F. (2009). Deletion of the alpha 7 nicotinic acetylcholine receptor gene improves cognitive deficits and synaptic pathology in a mouse model of Alzheimer's disease. J. Neurosci. 29, 8805-8815.

Eriksen J.L., Sagi S.A., Smith T.E., Weggen S., Das P., McLendon D.C., Ozols V.V., Jessing K.W., Zavitz K.H., Koo E.H. and Golde T.E. (2003). NSAIDs and enantiomers of flurbiprofen target gamma-secretase and lower Aß42 in vivo. J. Clin. Invest. 112, 440-449.

Farfara D., Trudler D., Segev-Amzaleg N., Galron R., Stein R. and Frenkel D. (2011). Gamma-secretase component presenilin is important for microglia ,-amyloid clearance. Ann. Neurol. 69, 170-180.

Fattoretti P., Balietti M., Casoli T., Giorgetti B., Di S.G., Bertoni-Freddari C., Lattanzio F. and Sensi S.L. (2010). Decreased numeric density of succinic dehydrogenase-positive mitochondria in CA1 pyramidal neurons of 3xTg-AD mice. Rejuvenation Res. 13, 144-147.

Faux N.G., Ritchie C.W., Gunn A., Rembach A., Tsatsanis A., Bedo J., Harrison J., Lannfelt L., Blennow K., Zetterberg H., Ingelsson M., Masters C.L., Tanzi R.E., Cummings J.L., Herd C.M. and Bush A.I. (2010). PBT2 rapidly improves cognition in Alzheimer's Disease: additional phase II analyses. J. Alzheimers Dis. 20, 509-516.

Flood D.G., Lin Y.G., Lang D.M., Trusko S.P., Hirsch J.D., Savage M.J., Scott R.W. and Howland D.S. (2009). A transgenic rat model of Alzheimer's disease with extracellular Aß deposition. Neurobiol. Aging 30, 1078-1090.

Fornai F., Longone P., Cafaro L., Kastsiuchenka O., Ferrucci M., Manca M.L., Lazzeri G., Spalloni A., Bellio N., Lenzi P., Modugno N., Siciliano G., Isicloro C., Murri L., Ruggieri S. and Paparelli A. (2008). Lithium delays progression of amyotrophic lateral sclerosis. Proc. Natl. Acad. Sci. USA 105, 2052-2057.

Forsell C., Bjork B.F., Lilius L., Axelman K., Fabre S.F., Fratiglioni L., Winblad B. and Graff C. (2010). Genetic association to the amyloid plaque associated protein gene COL25A1 in Alzheimer's disease. Neurobiol. Aging 31, 409-415.

Frazer M.E., Hughes J.E., Mastrangelo M.A., Tibbens J.L., Federoff H.J. and Bowers W.J. (2008). Reduced pathology and improved behavioral performance in Alzheimer's disease mice vaccinated with HSV amplicons expressing amyloid-beta and interleukin-4. Mol. Ther. 16, 845-853.

Fukumoto H., Takahashi H., Tarui N., Matsui J., Tomita T., Hirode M., Sagayama M., Maeda R., Kawamoto M., Hirai K., Terauchi J., Sakura Y., Kakihana M., Kato K., Iwatsubo T. and Miyamoto M. (2010). A noncompetitive BACE1 inhibitor TAK-070 ameliorates Aß pathology and behavioral deficits in a mouse model of Alzheimer's disease. J. Neurosci. 30, 11157-11166.

Gama Sosa M.A., Gasperi R.D., Rocher A.B., Wang A.C., Janssen W.G., Flores T., Perez G.M., Schmeidler J., Dickstein D.L., Hof P.R. and Elder G.A. (2010). Age-related vascular pathology in transgenic mice expressing presenilin 1-associated familial Alzheimer's disease mutations. Am. J. Pathol. 176, 353-368.

Games D., Adams D., Alessandrini R., Barbour R., Berthelette P., Blackwell C., Carr T., Clemens J., Donaldson T., Gillespie F., Guido T., Hagopian S., Johnsonwood K., Khan K., Lee M., Leibowitz P., Lieberburg I., Little S., Masliah E., McConlogue L., Montoyazavala M., Mucke L., Paganini L., Penniman E., Power M., Schenk D., Seubert P., Snyder B., Soriano F., Tan H., Vitale J., Wadsworth S., Wolozin B. and Zhao J. (1995). Alzheimer-type neuropathology in transgenic mice overexpressing v717f beta-amyloid precursor protein. Nature 373, 523-527.

Garcia-Alloza M., Borrelli L.A., Rozkalne A., Hyman B.T. and Bacskai B.J. (2007). Curcumin labels amyloid pathology in vivo, disrupts existing plaques, and partially restores distorted neurites in an Alzheimer mouse model. J. Neurochem. 102, 1095-1104.

Gauthier S., Aisen P.S., Ferris S.H., Saumier D., Duong A., Haine D., Garceau D., Suhy J., Oh J., Lau W. and Sampalis J. (2009). Effect of tramiprosate in patients with mild-to-moderate Alzheimer's disease: exploratory analyses of the MRI sub-group of the Alphase study. J.Nutr. Health Aging 13, 550-557.

Gervais F., Paquette J., Morissette C., Krzywkowski P., Yu M., Azzi M., Lacombe D., Kong X.Q., Aman A., Laurin J., Szarek W.A. and Tremblay P. (2007). Targeting soluble A beta peptide with Tramiprosate for the treatment of brain amyloidosis. Neurobiol. Aging 28, 537-547.

Glabe C.G. (2004). Conformation-dependent antibodies target diseases of protein misfolding. Trends Biochem. Sci. 29, 542-547.

Goate A., Chartier-Harlin M.C., Mullan M., Brown J., Crawford F., Fidani L., Giuffra L., Haynes A., Irving N. and James L. (1991). Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature 349, 704-706.

Goni F., Prelli F., Ji Y., Scholtzova H., Yang J., Sun Y., Liang F.X., Kascsak R., Kascsak R., Mehta P. and Wisniewski T. (2010). Immunomodulation targeting abnormal protein conformation reduces pathology in a mouse model of Alzheimer's disease. PLoS ONE 5, 10.1371/journal.pone.0013391.

Grathwohl S.A., Kalin R.E., Bolmont T., Prokop S., Winkelmann G., Kaeser S.A., Odenthal J., Radde R., Eldh T., Gandy S., Aguzzi A., Staufenbiel M., Mathews P.M., Wolburg H., Heppner F.L. and Jucker M. (2009). Formation and maintenance of Alzheimer's disease beta-amyloid plaques in the absence of microglia. Nat. Neurosci. 12, 1361-1363.

Guglielmotto M., Aragno M., Autelli R., Giliberto L., Novo E., Colombatto S., Danni O., Parola M., Smith M.A., Perry G., Tamagno E. and Tabaton M. (2009). The up-regulation of BACE1 mediated by hypoxia and ischemic injury: role of oxidative stress and HIF1alpha. J. Neurochem. 108, 1045-1056.

Haass C. and Selkoe D.J. (2007). Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. Nat. Rev. Mol. Cell Biol. 8, 101-112.

Hamaguchi T., Ono K., Murase A. and Yamada M. (2009). Phenolic compounds prevent Alzheimer's pathology through different effects on the amyloid-beta aggregation pathway. Am. J. Pathol. 175, 2557-2565.

Hamilton R.L. (2000). Lewy bodies in Alzheimer's disease: a neuropathological review of 145 cases using alpha-synuclein immunohistochemistry. Brain Pathol. 10, 378-384.

Hamilton L.K., Aumont A., Julien C., Vadnais A., Calon F. and

Fernandes K.J. (2010). Widespread deficits in adult neurogenesis precede plaque and tangle formation in the 3xTg mouse model of Alzheimer's disease. Eur. J. Neurosci. 32, 905-920.

Hampel H., Ewers M., Burger K., Annas P., Mortberg A., Bogstedt A., Frolich L., Schroder J., Schonknecht P., Riepe M.W., Kraft I., Gasser T., Leyhe T., Moller H.J., Kurz A. and Basun H. (2009). Lithium trial in Alzheimer's disease: a randomized, single-blind, placebo-controlled, multicenter 10-week study. J. Clin. Psychiatry 70, 922-931.

Hardy J. and Allsop D. (1991). Amyloid deposition as the central event in the aetiology of Alzheimer's disease. Trends. Pharmacol. Sci. 12, 383-388.

Hardy J. and Selkoe D.J. (2002). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 297, 353-356.

Hartig W., Goldhammer S., Bauer U., Wegner F., Wirths O., Bayer T.A. and Grosche J. (2010). Concomitant detection of beta-amyloid peptides with N-terminal truncation and different C-terminal endings in cortical plaques from cases with Alzheimer's disease, senile monkeys and triple transgenic mice. J. Chem. Neuroanat. 40, 82-92.

Hashimoto T., Wakabayashi T., Watanabe A., Kowa H., Hosoda R., Nakamura A., Kanazawa I., Arai T., Takio K., Mann D.M.A. and Iwatsubo T. (2002). CLAC: a novel Alzheimer amyloid plaque component derived from a transmembrane precursor, CLAC-P/collagen type XXV. EMBO J. 21, 1524-1534.

Head E., Pop V., Sarsoza F., Kayed R., Beckett T.L., Studzinski C.M., Tomic J.L., Glabe C.G. and Murphy M.P. (2010). Amyloid-beta peptide and oligomers in the brain and cerebrospinal fluid of aged canines. J. Alzheimers Dis. 20, 637-646.

Henderson V.W. (2008). Cognitive changes after menopause: influence of estrogen. Clin. Obst. Gynecol. 51, 618-626.

Herber D.L., Mercer M., Roth L.M., Symmonds K., Maloney J., Wilson N., Freeman M.J., Morgan D. and Gordon M.N. (2007). Microglial activation is required for Aß clearance after intracranial injection of lipopolysaccharide in APP transgenic mice. J. Neuroimmune Pharmacol. 2, 222-231.

Hermann D., Both M., Ebert U., Gross G., Schoemaker H., Draguhn A., Wicke K. and Nimmrich V. (2009). Synaptic transmission is impaired prior to plaque formation in amyloid precursor protein-overexpressing mice without altering behaviorally-correlated sharp wave-ripple complexes. Neuroscience 162, 1081-1090.

Hernandez C.M., Kayed R., Zheng H., Sweatt J.D. and Dineley K.T. (2010). Loss of alpha7 nicotinic receptors enhances beta-amyloid oligomer accumulation, exacerbating early-stage cognitive decline and septohippocampal pathology in a mouse model of Alzheimer's disease. J. Neurosci. 30, 2442-2453.

Hiesberger T., Trommsdorff M., Howell B.W., Goffinet A., Mumby M.C., Cooper J.A. and Herz J. (1999). Direct binding of Reelin to VLDL receptor and ApoE receptor 2 induces tyrosine phosphorylation of disabled-1 and modulates tau phosphorylation. Neuron 24, 481-489.

Holmes C., Boche D., Wilkinson D., Yadegarfar G., Hopkins V., Bayer A., Jones R.W., Bullock R., Love S., Neal J.W., Zotova E. and Nicoll J.A. (2008). Long-term effects of Aß42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. Lancet 372, 216-223.

Hook V.Y., Kindy M. and Hook G. (2008). Inhibitors of cathepsin B improve memory and reduce beta-amyloid in transgenic Alzheimer disease mice expressing the wild-type, but not the Swedish mutant, beta-secretase site of the amyloid precursor protein. J. Biol. Chem. 283, 7745-7753.

Houeland G., Romani A., Marchetti C., Amato G., Capsoni S., Cattaneo A. and Marie H. (2010). Transgenic mice with chronic NGF deprivation and Alzheimer's disease-like pathology display hippocampal region-specific impairments in short- and long-term plasticities. J. Neurosci. 30, 13089-13094.

Howlett D.R. and Richardson J.C. (2009). The pathology of APP transgenic mice: a model of Alzheimer's disease or simply overexpression of APP? Histol. Histopathol. 24, 83-100.

Howlett D.R., Richardson J.C., Austin A., Parsons A.A., Bate S.T., Davies D.C. and Gonzalez M.I. (2004). Cognitive correlates of A beta deposition in male and female mice bearing amyloid precursor protein and presenilin-1 mutant transgenes. Brain Res. 1017, 130-136.

Howlett D.R., Bowler K., Soden P.E., Riddell D., Davis J.B., Richardson J.C., Burbidge S.A., Gonzalez M.I., Irving E.A., Lawman A., Miglio G., Dawson E.L., Howlett E.R. and Hussain I. (2008). Aß deposition and related pathology in an APP x PS1 transgenic mouse model of Alzheimer's disease. Histol. Histopathol. 23, 67-76.

Hsiao K., Chapman P., Nilsen S., Eckman C., Harigaya Y., Younkin S., Yang F. and Cole G. (1996). Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. Science 274, 99-102.

Hu Y.S., Xu P., Pigino G., Brady S.T., Larson J. and Lazarov O. (2010). Complex environment experience rescues impaired neurogenesis, enhances synaptic plasticity, and attenuates neuropathology in familial Alzheimer's disease-linked APPswe/PS1DeltaE9 mice. FASEB J. 24, 1667-1681.

Hudry E., Van Dam D., Kulik W., De Deyn P.P., Stet F.S., Ahouansou O., Benraiss A., Delacourte A., Bougneres P., Aubourg P. and Cartier N. (2010). Adeno-associated virus gene therapy with cholesterol 24-hydroxylase reduces the amyloid pathology before or after the onset of amyloid plaques in mouse models of Alzheimer's disease. Mol. Ther. 18, 44-53.

Hussain I. (2010). APP transgenic mouse models and their use in drug discovery to evaluate amyloid- lowering therapeutics. CNS Neurol. Disord. Drug Targets 9, 395-402.

Hussain I., Powell D., Howlett D.R., Tew D.G., Meek T.D., Chapman C., Gloger I.S., Murphy K.E., Southan C.D., Ryan D.M., Smith T.S., Simmons D.L., Walsh F.S., Dingwall C. and Christie G. (1999). Identification of a novel aspartic protease (Asp 2) as beta-secretase. Mol. Cell. Neurosci. 14, 419-427.

Hutter-Paier B., Huttunen H.J., Puglielli L., Eckman C.B., Kim D.Y., Hofmeister A., Moir R.D., Domnitz S.B., Frosch M.P., Windisch M. and Kovacs D.M. (2004). The ACAT inhibitor CP-113,818 markedly reduces amyloid pathology in a mouse model of Alzheimer's disease. Neuron 44, 227-238.

Huttunen H.J., Havas D., Peach C., Barren C., Duller S., Xia W., Frosch M.P., Hutter-Paier B., Windisch M. and Kovacs D.M. (2010). The acyl-coenzyme A: cholesterol acyltransferase inhibitor CI-1011 reverses diffuse brain amyloid pathology in aged amyloid precursor protein transgenic mice. J. Neuropathol. Exp. Neurol. 69, 777-788.

Imbimbo B.P. (2009). Why did tarenflurbil fail in Alzheimer's disease? J. Alzheimers Dis. 17, 757-760.

Imbimbo B.P., Giardino L., Sivilia S., Giuliani A., Gusciglio M., Pietrini V., Del G.E., D'Arrigo A., Leon A., Villetti G. and Calza L. (2010). CHF5074, a novel gamma-secretase modulator, restores hippocampal neurogenesis potential and reverses contextual memory deficit in a transgenic mouse model of Alzheimer's disease.

J. Alzheimers Dis. 20, 159-173.

Iwatsubo T., Mann D.M.A., Odaka A., Suzuki N. and Ihara Y. (1995). Amyloid-beta protein (a-beta) deposition - a-beta-42(43) precedes a-beta-40 in Down-syndrome. Ann. Neurol. 37, 294-299.

Jankowsky J.L., Slunt H.H., Gonzales V., Jenkins N.A., Copeland N.G. and Borchelt D.R. (2004). APP processing and amyloid deposition in mice haplo-insufficient for presenilin 1. Neurobiol. Aging 25, 885-892.

Janus C., Pearson J., McLaurin J., Mathews P.M., Jiang Y., Schmidt S.D., Chishti M.A., Horne P., Heslin D., French J., Mount H.T., Nixon R.A., Mercken M., Bergeron C., Fraser P.E., George-Hyslop P. and Westaway D. (2000). A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. Nature 408, 979-982.

Jolivalt C.G., Hurford R., Lee C.A., Dumaop W., Rockenstein E. and Masliah E. (2010). Type 1 diabetes exaggerates features of Alzheimer's disease in APP transgenic mice. Exp. Neurol. 223, 422-431.

Kakuyama H., Soderberg L., Horigome K., Winblad B., Dahlqvist C., Naslund J. and Tjernberg L.O. (2005). CLAC binds to aggregated A beta and A beta fragments, and attenuates fibril elongation. Biochemistry 44, 15602-15609.

Kalback W., Watson M.D., Kokjohn T.A., Kuo Y.M., Weiss N., Luehrs D.C., Lopez J., Brune D., Sisodia S.S., Staufenbiel M., Emmerling M. and Roher A.E. (2002). APP transgenic mice Tg2576 accumulate A beta peptides that are distinct from the chemically modified and insoluble peptides deposited in Alzheimer's disease senile plaques. Biochemistry 41, 922-928.

Kang C.K., Jayasinha V. and Martin P.T. (2003). Identification of peptides that specifically bind Aß1-40 amyloid in vitro and amyloid plaques in Alzheimer's disease brain using phage display. Neurobiol. Dis. 14, 146-156.

Karlnoski R., Wilcock D., Dickey C., Ronan V., Gordon M.N., Zhang W., Morgan D. and Taglialatela G. (2007). Up-regulation of Bcl-2 in APP transgenic mice is associated with neuroprotection. Neurobiol. Dis. 25, 179-188.

Kitaguchi H., Tomimoto H., Ihara M., Shibata M., Uemura K., Kalaria R.N., Kihara T., sada-Utsugi M., Kinoshita A. and Takahashi R. (2009). Chronic cerebral hypoperfusion accelerates amyloid beta deposition in APPSwInd transgenic mice. Brain Res. 1294, 202-210.

Knafo S., Alonso-Nanclares L., Gonzalez-Soriano J., Merino-Serrais P., Fernaud-Espinosa I., Ferrer I. and DeFelipe J. (2009a). Widespread changes in dendritic spines in a model of Alzheimer's disease. Cereb. Cortex 19, 586-592.

Knafo S., Venero C., Merino-Serrais P., Fernaud-Espinosa I., Gonzalez-Soriano J., Ferrer I., Santpere G., DeFelipe J., Knafo S., Venero C., Merino-Serrais P., Fernaud-Espinosa I., Gonzalez-Soriano J., Ferrer I., Santpere G. and DeFelipe J. (2009b). Morphological alterations to neurons of the amygdala and impaired fear conditioning in a transgenic mouse model of Alzheimer's disease. J. Pathol. 219, 41-51.

Kocherhans S., Madhusudan A., Doehner J., Breu K.S., Nitsch R.M., Fritschy J.M. and Knuesel I. (2010). Reduced Reelin expression accelerates amyloid-beta plaque formation and tau pathology in transgenic Alzheimer's disease mice. J. Neurosci. 30, 9228-9240.

Koenigsknecht-Talboo J., Meyer-Luehmann M., Parsadanian M., Garcia-Alloza M., Finn M.B., Hyman B.T., Bacskai B.J. and Holtzman D.M. (2008). Rapid microglial response around amyloid pathology after systemic anti-Aß antibody administration in PDAPP mice. J. Neurosci. 28, 14156-14164.

Kounnas M.Z., Danks A.M., Cheng S., Tyree C., Ackerman E., Zhang X., Ahn K., Nguyen P., Comer D., Mao L., Yu C., Pleynet D., Digregorio P.J., Velicelebi G., Stauderman K.A., Comer W.T., Mobley W.C., Li Y.M., Sisodia S.S., Tanzi R.E. and Wagner S.L. (2010). Modulation of gamma-secretase reduces beta-amyloid deposition in a transgenic mouse model of Alzheimer's disease. Neuron 67, 769-780.

Kuo Y.M., Emmerling M.R., Woods A.S., Cotter R.J. and Roher A.E. (1997). Isolation, chemical characterization, and quantitation of A beta 3-pyroglutamyl peptide from neuritic plaques and vascular amyloid deposits. . Biochem. Biophys. Res. Commun. 237, 188-191.

Kuo Y.M., Kokjohn T.A., Beach T.G., Sue L.I., Brune D., Lopez J.C., Kalback W.M., Abramowski D., Sturchler-Pierrat C., Staufenbiel M. and Roher A.E. (2001). Comparative analysis of amyloid-beta chemical structure and amyloid plaque morphology of transgenic mouse and Alzheimer's disease brains. J. Biol. Chem. 276, 12991-12998.

Lee K.W., Kim J.B., Seo J.S., Kim T.K., Im J.Y., Baek I.S., Kim K.S., Lee J.K. and Han P. L. (2009). Behavioral stress accelerates plaque pathogenesis in the brain of Tg2576 mice via generation of metabolic oxidative stress. J. Neurochem. 108, 165-175.

Lee D.C., Rizer J., Selenica M.L., Reid P., Kraft C., Johnson A., Blair L., Gordon M.N., Dickey C.A. and Morgan D. (2010a). LPS-induced inflammation exacerbates phospho-tau pathology in rTg4510 mice. J. Neuroinflamm. 7, 56.

Lee J.H., Won S.M., Suh J., Son S.J., Moon G.J., Park U.J. and Gwag B.J. (2010b). Induction of the unfolded protein response and cell death pathway in Alzheimer's disease, but not in aged Tg2576 mice. Exp. Mol. Med. 42, 386-394.

Lee S., Varvel N.H., Konerth M.E., Xu G., Cardona A.E., Ransohoff R.M. and Lamb B.T. (2010c). CX3CR1 deficiency alters microglial activation and reduces beta-amyloid deposition in two Alzheimer's disease mouse models. Am. J. Pathol. 177, 2549-2562.

Leibson C.L., Rocca W.A., Hanson V.A., Cha R., Kokmen E., O'Brien P.C. and Palumbo P.J. (1997). Risk of dementia among persons with diabetes mellitus: a population-based cohort study. Am. J. Epidemiol. 145, 301-308.

Lemere C.A., Beierschmitt A., Iglesias M., Spooner E.T., Bloom J.K., Leverone J.F., Zheng J.B., Seabrook T.J., Louard D., Li D., Selkoe D.J., Palmour R.M. and Ervin F.R. (2004). Alzheimer's disease A beta vaccine reduces central nervous system A beta levels in a non-human primate, the Caribbean Vervet. Am. J. Pathol. 165, 283-297.

Lemere C.A., Oh J., Stanish H.A., Peng Y., Pepivani I., Fagan A.M., Yamaguchi H., Westmoreland S.V. and Mansfield K.G. (2008). Cerebral amyloid-beta protein accumulation with aging in cotton-top tamarins: A model of early Alzheimer's disease? Rejuvenation Res. 11, 321-332.

Leon W.C., Canneva F., Partridge V., Allard S., Ferretti M.T., DeWilde A., Vercauteren F., Atifeh R., Ducatenzeiler A., Klein W., Szyf M., Alhonen L. and Cuello A.C. (2010). A novel transgenic rat model with a full Alzheimer's-like amyloid pathology displays pre-plaque intracellular amyloid-beta-associated cognitive impairment. J. Alzheimers Dis. 20, 113-126.

Leroy K., Ando K., Heraud C., Yilmaz Z., Authelet M., Boeynaems J.M., Buee L., De Decker R. and Brion J.P. (2010). Lithium treatment arrests the development of neurofibrillary tangles in mutant tau transgenic mice with advanced neurofibrillary pathology. J. Alzheimers Dis. 19, 705-719.

Lim G.P., Yang F., Chu T., Chen P., Beech W., Teter B., Tran T., Ubeda O., Ashe K.H., Frautschy S.A. and Cole G.M. (2000). Ibuprofen suppresses plaque pathology and inflammation in a mouse model for Alzheimer's disease. J. Neurosci. 20, 5709-5714.

Liu L., Orozco I.J., Planel E., Wen Y., Bretteville A., Krishnamurthy P., Wang L., Herman M., Figueroa H., Yu W.H., Arancio O. and Duff K. (2008a). A transgenic rat that develops Alzheimer's disease-like amyloid pathology, deficits in synaptic plasticity and cognitive impairment. Neurobiol. Dis. 31, 46-57.

Liu Y., Yoo M.J., Savonenko A., Stirling W., Price D.L., Borchelt D.R., Mamounas L., Lyons W.E., Blue M.E. and Lee M.K. (2008b). Amyloid pathology is associated with progressive monoaminergic neurodegeneration in a transgenic mouse model of Alzheimer's disease. J. Neurosci. 28, 13805-13814.

Liu B., Rasool S., Yang Z., Glabe C.G., Schreiber S.S., Ge J. and Tan Z. (2009). Amyloid-peptide vaccinations reduce beta-amyloid plaques but exacerbate vascular deposition and inflammation in the retina of Alzheimer's transgenic mice. Am. J. Pathol. 175, 2099-2110.

Liu Q., Trotter J., Zhang J., Peters M.M., Cheng H., Bao J., Han X., Weeber E.J. and Bu G. (2010a). Neuronal LRP1 knockout in adult mice leads to impaired brain lipid metabolism and progressive, age-dependent synapse loss and neurodegeneration. J. Neurosci. 30, 17068-17078.

Liu Z., Condello C., Schain A., Harb R. and Grutzendler J. (2010b). CX3CR1 in microglia regulates brain amyloid deposition through selective protofibrillar amyloid-phagocytosis. J. Neurosci. 30, 17091-17101.

Lleo A., Berezovska O., Growdon J.H. and Hyman B.T. (2004). Clinical, pathological, and biochemical spectrum of Alzheimer disease associated with PS-1 mutations. Am. J. Geriatr. Psychiatry 12, 146-156.

Ma Q.L., Yang F., Rosario E.R., Ubeda O.J., Beech W., Gant D.J., Chen P.P., Hudspeth B., Chen C., Zhao Y., Vinters H.V., Frautschy S.A. and Cole G.M. (2009). Beta-amyloid oligomers induce phosphorylation of tau and inactivation of insulin receptor substrate via c-Jun N-terminal kinase signaling: suppression by omega-3 fatty acids and curcumin. J. Neurosci. 29, 9078-9089.

Mancuso C., Siciliano R. and Barone E. (2011). Curcumin and Alzheimer disease: this marriage is not to be performed. J. Biol. Chem. 286, le3.

Martone R.L., Zhou H., Atchison K., Comery T., Xu J.Z., Huang X., Gong X., Jin M., Kreft A., Harrison B., Mayer S.C., Aschmies S., Gonzales C., Zaleska M.M., Riddell D.R., Wagner E., Lu P., Sun S.C., Sonnenberg-Reines J., Oganesian A., Adkins K., Leach M.W., Clarke D.W., Huryn D., Abou-Gharbia M., Magolda R., Bard J., Frick G., Raje S., Forlow S.B., Balliet C., Burczynski M.E., Reinhart P.H., Wan H.I., Pangalos M.N. and Jacobsen J.S. (2009). Begacestat (GSI-953): a novel, selective thiophene sulfonamide inhibitor of amyloid precursor protein gamma-secretase for the treatment of Alzheimer's disease. J. Pharm. Exp. Ther. 331, 598-608.

Masliah E., Rockenstein E., Veinbergs I., Sagara Y., Mallory M., Hashimoto M. and Mucke L. (2001). beta-Amyloid peptides enhance alpha-synuclein accumulation and neuronal deficits in a transgenic mouse model linking Alzheimer's disease and Parkinson's disease. Proc. Natl. Acad. Sci. USA 98, 12245-12250.

Mastrangelo M.A., Sudol K.L., Narrow W.C. and Bowers W.J. (2009). Interferon-gamma differentially affects Alzheimer's disease pathologies and induces neurogenesis in triple transgenic-AD mice. Am. J. Pathol. 175, 2076-2088.

McAlpine F.E., Lee J.K., Harms A.S., Ruhn K.A., Blurton-Jones M., Hong J., Das P., Golde T.E., LaFerla F.M., Oddo S., Blesch A. and Tansey M.G. (2009). Inhibition of soluble TNF signaling in a mouse model of Alzheimer's disease prevents pre-plaque amyloid-associated neuropathology. Neurobiol. Dis. 34, 163-177.

McConlogue L., Buttini M., Anderson J.P., Brigham E.F., Chen K.S., Freedman S.B., Games D., Johnson-Wood K., Lee M., Zeller M., Liu W., Motter R. and Sinha S. (2007). Partial reduction of BACE1 has dramatic effects on Alzheimer plaque and synaptic pathology in APP Transgenic Mice. J. Biol. Chem. 282, 26326-26334.

McGeer P.L., Schulzer M. and McGeer E.G. (1996). Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies. Neurology 47, 425-432.

McLaurin J., Kierstead M.E., Brown M.E., Hawkes C.A., Lambermon M.H., Phinney A.L., Darabie A.A., Cousins J.E., French J.E., Lan M.F., Chen F., Wong S.S., Mount H.T., Fraser P.E., Westaway D. and George-Hyslop P. (2006). Cyclohexanehexol inhibitors of Aß aggregation prevent and reverse Alzheimer phenotype in a mouse model. Nat. Med. 12, 801-808.

Meadowcroft M.D., Connor J.R., Smith M.B. and Yang Q.X. (2009). MRI and histological analysis of beta-amyloid plaques in both human Alzheimer's disease and APP/PS1 transgenic mice. J. Magn. Reson. Imaging 29, 997-1007.

Mei X., Ezan P., Giaume C. and Koulakoff A. (2010). Astroglial connexin immunoreactivity is specifically altered at ,-amyloid plaques in ,-amyloid precursor protein/presenilin1 mice. Neuroscience 171, 92-105.

Meyer-Luehmann M., Spires-Jones T.L., Prada C., Garcia-Alloza M., De Calignon A., Rozkalne A., Koenigsknecht-Talboo J., Holtzman D.M., Bacskai B.J. and Hyman B.T. (2008). Rapid appearance and local toxicity of amyloid-beta plaques in a mouse model of Alzheimer's disease. Nature 451, 720-724

Moechars D., Dewachter I., Lorent K., Reverse D., Baekelandt V., Naidu A., Tesseur I., Spittaels K., Van den Haute C.V., Checler F., Godaux E., Cordell B. and Van Leuven F. (1999). Early phenotypic changes in transgenic mice that overexpress different mutants of amyloid precursor protein in brain. J. Biol. Chem. 274, 6483-6492.

Morales R., Estrada L.D., az-Espinoza R., Morales-Scheihing D., Jara M.C., Castilla J. and Soto C. (2010). Molecular cross talk between misfolded proteins in animal models of Alzheimer's and prion diseases. J. Neurosci. 30, 4528-4535.

Morgan D., Diamond D.M., Gottschall P.E., Ugen K.E., Dickey C., Hardy J., Duff K., Jantzen P., DiCarlo G., Wilcock D., Connor K., Hatcher J., Hope C., Gordon M. and Arendash G.W. (2000). A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. Nature 408, 982-985.

Mori K. (2000). Tripartite management of unfolded proteins in the endoplasmic reticulum. Cell 101, 451-454.

Mori T., Paris D., Town T., Rojiani A.M., Sparks D.L., Delledonne A., Crawford F., Abdullah L.I., Humphrey J.A., Dickson D.W. and Mullan M.J. (2001). Cholesterol accumulates in senile plaques of Alzheimer disease patients and in transgenic APP(SW) mice. J. Neuropathol. Exp. Neurol. 60, 778-785.

Mori T., Koyama N., Arendash G.W., Horikoshi-Sakuraba Y., Tan J. and Town T. (2010). Overexpression of human S100B exacerbates cerebral amyloidosis and gliosis in the Tg2576 mouse model of Alzheimer's disease. Glia 58, 300-314.

Morrissette D.A., Parachikova A., Green K.N. and Laferla F.M. (2009). Relevance of transgenic mouse models to human Alzheimer disease. J. Biol. Chem. 284, 6033-6037.

Mouri A., Noda Y., Hara H., Mizoguchi H., Tabira T. and Nabeshima T. (2007). Oral vaccination with a viral vector containing Aß cDNA attenuates age-related Aß accumulation and memory deficits without causing inflammation in a mouse Alzheimer model. FASEB J. 21, 2135-2148.

Movsesyan N., Ghochikyan A., Mkrtichyan M., Petrushina I., Davtyan H., Olkhanud P.B., Head E., Biragyn A., Cribbs D.H. and Agadjanyan M.G. (2008). Reducing AD-like pathology in 3xTg-AD mouse model by DNA epitope vaccine - a novel immunotherapeutic strategy. PLoS ONE 3, e2124

Mucke L., Masliah E., Yu G.Q., Mallory M., Rockenstein E.M., Tatsuno G., Hu K., Kholodenko D., Johnson-Wood K., and McConlogue L. (2000). High-level neuronal expression of Abeta 1-42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. J. Neurosci. 20, 4050-4058.

Muhs A., Hickman D.T., Pihlgren M., Chuard N., Giriens V., Meerschman C., Van der Auwera I., Van Leuven F., Sugawara M., Weingertner M.C., Bechinger B., Greferath R., Kolonko N., Nagel-Steger L., Riesner D., Brady R.O., Pfeifer A. and Nicolau C. (2007). Liposomal vaccines with conformation-specific amyloid peptide antigens define immune response and efficacy in APP transgenic mice. Proc. Natl. Acad. Sci. USA 104, 9810-9815.

Nicoll J.A.R., Wilkinson D., Holmes C., Steart P., Markham H. and Weller R.O. (2003). Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report. Nat. Med. 9, 448-452.

Nitsch R.M., Slack B.E., Wurtman R.J. and Growdon J.H. (1992). Release of Alzheimer amyloid precursor derivatives stimulated by activation of muscarinic acetylcholine receptors. Science 258, 304-307.

Nixon R.A., Cataldo A.M. and Mathews P.M. (2000). The endosomal-lysosomal system of neurons in Alzheimer's disease pathogenesis: A review. Neurochem. Res. 25, 1161-1172.

Noble W., Hanger D.P. and Gallo J.M. (2010). Transgenic mouse models of tauopathy in drug discovery. CNS Neurol. Disord. Drug Targets 9, 403-428.

Nordberg A., Hellstrom-Lindahl E., Lee M., Johnson M., Mousavi M., Hall R., Perry E., Bednar I. and Court J. (2002). Chronic nicotine treatment reduces beta-amyloidosis in the brain of a mouse model of Alzheimer's disease (APPsw). J. Neurochem. 81, 655-658.

Oakley H., Cole S.L., Logan S., Maus E., Shao P., Craft J., Guillozet-Bongaarts A., Ohno M., Disterhoft J., Van Eldik L., Berry R. and Vassar R. (2006). Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: Potential factors in amyloid plaque formation. J. Neurosci. 26, 10129-10140.

Oddo S., Caccamo A., Shepherd J.D., Murphy M.P., Golde T.E., Kayed R., Metherate R., Mattson M.P., Akbari Y. and Laferla F.M. (2003). Triple-transgenic model of Alzheimer's disease with plaques and tangles: Intracellular A beta and synaptic dysfunction. Neuron 39, 409-421.

Oddo S., Billings L., Kesslak J.P., Cribbs D.H. and Laferla F.M. (2004). A beta immunotherapy leads to clearance of early, but not late, hyperphosphorylated tau aggregates via the proteasome. Neuron 43, 321-332.

Overk C.R., Kelley C.M. and Mufson E.J. (2009). Brainstem Alzheimer's-like pathology in the triple transgenic mouse model of Alzheimer's disease. Neurobiol. Dis. 35, 415-425.

Palop J.J., Chin J., Roberson E.D., Wang J., Thwin M.T., Bien-Ly N., Yoo J., Ho K.O., Yu G.Q., Kreitzer A., Finkbeiner S., Noebels J.L. and Mucke L. (2007). Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. Neuron 55, 697-711.

Paquet D., Schmid B. and Haass C. (2010). Transgenic zebrafish as a novel animal model to study tauopathies and other neurodegenerative disorders in vivo. Neurodegen. Dis. 7, 99-102.

Parachikova A., Vasilevko V., Cribbs D.H., Laferla F.M. and Green K.N. (2010). Reductions in amyloid-beta-derived neuroinflammation, with minocycline, restore cognition but do not significantly affect tau hyperphosphorylation. J. Alzheimers Dis. 21, 527-542.

Paris D., Ganey N.J., Laporte V., Patel N.S., Beaulieu-Abdelahad D., Bachmeier C., March A., it-Ghezala G. and Mullan M.J. (2010). Reduction of beta-amyloid pathology by celastrol in a transgenic mouse model of Alzheimer's disease. J. Neuroinflamm. 7, 17.

Pasqualetti P., Bonomini C., Dal Forno G., Paulon L., Sinforiani E., Marra C., Zanetti O. and Rossini P.M. (2009). A randomized controlled study on effects of ibuprofen on cognitive progression of Alzheimer's disease. Aging Clin. Exp. Res. 21, 102-10.

Perez S.E., Berg B.M., Moore K.A., He B., Counts S.E., Fritz J.J., Hu Y.S., Lazarov O., Lah J.J. and Mufson E.J. (2010). DHA diet reduces AD pathology in young APPswe/PS1 Delta E9 transgenic mice: possible gender effects. J. Neurosci. Res. 88, 1026-1040.

Pericak-Vance M.A., Bebout J.L., Gaskell P.C. Jr, Yamaoka L.H., Hung W.Y., Alberts M.J., Walker A.P., Bartlett R.J., Haynes C.A. and Welsh K.A. (1991). Linkage studies in familial Alzheimer disease: evidence for chromosome 19 linkage. Am. J. Hum. Genet. 48, 1034-1050.

Permanne B., Adessi C., Saborio G.P., Fraga S., Frossard M.J., Van Dorpe J., Dewachter I., Banks W.A., Van Leuven F. and Soto C. (2002). Reduction of amyloid load and cerebral damage in a transgenic mouse model of Alzheimer's disease by treatment with a beta-sheet breaker peptide. FASEB J. 16, 860-862.

Philipson O., Hammarstrom P., Nilsson K.P., Portelius E., Olofsson T., Ingelsson M., Hyman B.T., Blennow K., Lannfelt L., Kalimo H. and Nilsson L.N.G. (2009). A highly insoluble state of Aß similar to that of Alzheimer's disease brain is found in Arctic APP transgenic mice. Neurobiol. Aging 30, 1393-1405.

Puglielli L., Tanzi R.E. and Kovacs D.M. (2003). Alzheimer's disease: the cholesterol connection. Nat. Neurosci. 6, 345-351.

Raina A.K., Hochman A., Zhu X.W., Rottkamp C.A., Nunomura A., Siedlak S.L., Boux H., Castellani R.J., Perry G. and Smith M.A. (2001). Abortive apoptosis in Alzheimer's disease. Acta Neuropathol. 101, 305-310.

Raina A.K., Hochman A., Ickes H., Zhu X.W., Ogawa O., Cash A.D., Shimohama S., Perry G. and Smith M.A. (2003). Apoptotic promoters and inhibitors in Alzheimer's disease: Who wins out? Prog. Neuropsychopharmacol. Biol. Psychiatry 27, 251-254.

Reines S.A., Block G.A., Morris J.C., Liu G., Nessly M.L., Lines C.R., Norman B.A. and Baranak C.C. (2004). Rofecoxib: no effect on Alzheimer's disease in a 1-year, randomized, blinded, controlled study. Neurology 62, 66-71.

Roberts G.W., Allsop D. and Bruton C. (1990). The occult aftermath of boxing. J. Neurol. Neurosurg. Psychiatry 53, 373-378.

Roberts G.W., Gentleman S.M., Lynch A., Murray L., Landon M. and Graham D.I. (1994). Beta amyloid protein deposition in the brain

after severe head injury: implications for the pathogenesis of Alzheimer's disease. J. Neurol. Neurosurg. Psychiatry 57, 419-425.

Rodriguez-Navarro J.A., Rodriguez L., Casarejos M.J., Solano R.M., Gomez A., Perucho J., Cuervo A.M., Garcia de Y.J. and Mena M.A. (2010). Trehalose ameliorates dopaminergic and tau pathology in parkin deleted/tau overexpressing mice through autophagy activation. Neurobiol. Dis. 39, 423-438.

Rohn T.T., Vyas V., Hernandez-Estrada T., Nichol K.E., Christie L.A. and Head E. (2008). Lack of pathology in a triple transgenic mouse model of Alzheimer's disease after overexpression of the anti-apoptotic protein Bcl-2. J. Neurosci. 28, 3051-3059.

Rosario E.R. and Pike C.J. (2008). Androgen regulation of beta-amyloid protein and the risk of Alzheimer's disease. Brain Res. Rev. 57, 444-453.

Rosario E.R., Carroll J.C., Oddo S., Laferla F.M. and Pike C.J. (2006). Androgens regulate the development of neuropathology in a triple transgenic mouse model of Alzheimer's disease. J. Neurosci. 26, 13384-13389.

Rosario E.R., Carroll J. and Pike C.J. (2010). Testosterone regulation of Alzheimer-like neuropathology in male 3xTg-AD mice involves both estrogen and androgen pathways. Brain Res. 1359, 281-290.

Ryu J.K., Cho T., Choi H.B., Wang Y.T. and McLarnon J.G. (2009). Microglial VEGF receptor response is an integral chemotactic component in Alzheimer's disease pathology. J. Neurosci. 29, 3-13.

Saido T.C., Iwatsubo T., Mann D.M., Shimada H., Ihara Y. and Kawashima S. (1995). Dominant and differential deposition of distinct beta-amyloid peptide species, A beta N3(pE), in senile plaques. Neuron 14, 457-466.

Satou T., Cummings B.J. and Cotman C.W. (1995). Immunoreactivity for Bcl-2 protein within neurons in the Alzheimer's disease brain increases with disease severity. Brain Res. 697, 35-43.

Saumier D., Aisen P.S., Gauthier S., Vellas B., Ferris S.H., Duong A., Suhy J., Oh J., Lau W., Garceau D., Haine D. and Sampalis J. (2009). Lessons learned in the use of volumetric MRI in therapeutic trials in Alzheimer's disease: the ALZHEMED (Tramiprosate) experience. J. Nutr. Health Aging 13, 370-372.

Schenk D., Barbour R., Dunn W., Gordon G., Grajeda H., Guido T., Hu K., Huang J.P., Johnson-Wood K., Khan K., Kholodenko D., Lee M., Liao Z.M., Lieberburg I., Motter R., Mutter L., Soriano F., Shopp G., Vasquez N., Vandevert C., Walker S., Wogulis M., Yednock T., Games D. and Seubert P. (1999). Immunization with amyloid-beta attenuates Alzheimer disease-like pathology in the PDAPP mouse. Nature 400, 173-177.

Schilling S., Zeitschel U., Hoffmann T., Heiser U., Francke M., Kehlen A., Holzer M., Hutter-Paier B., Prokesch M., Windisch M., Jagla W., Schlenzig D., Lindner C., Rudolph T., Reuter G., Cynis H., Montag D., Demuth H.U. and Rossner S. (2008). Glutaminyl cyclase inhibition attenuates pyroglutamate Aß and Alzheimer's disease-like pathology. Nat. Med. 14, 1106-1111.

Schneeberger A., Mandler M., Otawa O., Zauner W., Mattner F. and Schmidt W. (2009). Development of AFFITOPE vaccines for Alzheimer's disease (AD)--from concept to clinical testing. J. Nutr.Health Aging 13, 264-267.

Searfoss G.H., Jordan W.H., Calligaro D.O., Galbreath E.J., Schirtzinger L.M., Berridge B.R., Gao H., Higgins M.A., May P.C. and Ryan T.P. (2003). Adipsin, a biomarker of gastrointestinal toxicity mediated by a functional gamma-secretase inhibitor. J. Biol. Chem. 278, 46107-46116.

Senior K. (2002). Dosing in phase II trial of Alzheimer's vaccine suspended. Lancet Neurology 1, 3.

Sherrington R., Rogaev E.I., Liang Y., Rogaeva E.A., Levesque G., Ikeda M., Chi H., Lin C., Li G. and Holman K. (1995). Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. Nature 375, 754-760.

Siemers E., Skinner M., Dean R.A., Gonzales C., Satterwhite J., Farlow M., Ness D. and May P.C. (2005). Safety, tolerability, and changes in amyloid beta concentrations after administration of a gamma-secretase inhibitor in volunteers. Clin. Neuropharmacol. 28, 126-132.

Sigurdsson E.M. (2008). Immunotherapy targeting pathological tau protein in Alzheimer's disease and related tauopathies. J. Alzheimers Dis. 15, 157-168.

Sinha S., Anderson J.P., Barbour R., Basi G.S., Caccavello R., Davis D., Doan M., Dovey H.F., 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., Suomensaari S.M., Wang S.W., Walker D. and Zhao J. (1999). Purification and cloning of amyloid precursor protein beta-secretase from human brain. Nature 402, 537-540.

Small D.H., Maksel D., Kerr M.L., NG J., Hou X., Chu C., Mehrani H., Unabia S., Azari M.F., Loiacono R., Aguilar M.I. and Chebib M. (2007). The beta-amyloid protein of Alzheimer's disease binds to membrane lipids but does not bind to the alpha 7 nicotinic acetylcholine receptor. J. Neurochem. 101, 1527-1538.

Smith D.L., Pozueta J., Gong B., Arancio O. and Shelanski M. (2009). Reversal of long-term dendritic spine alterations in Alzheimer disease models. Proc. Natl. Acad. Sci. USA 106, 16877-16882.

Sonnen J.A., Larson E.B., Walker R.L., Haneuse S., Crane P.K., Gray S L., Breitner J.C.S. and Montine T.J. (2010). Nonsteroidal anti-inflammatory drugs are associated with increased neuritic plaques. Neurology 75, 1203-10.

Sturchler Pierrat C., Abramowski D., Duke M., Wiederhold K.H., Mistl C., Rothacher S., Ledermann B., Burki K., Frey P., Paganetti P.A., Waridel C., Calhoun M.E., Jucker M., Probst A., Staufenbiel M. and Sommer B. (1997). Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. Proc. Natl. Acad. Sci. USA 94, 13287-13292.

Sung S., Yang H.X., Uryu K., Lee E.B., Zhao L., Shineman D., Trojanowski J.Q., Lee V.M.Y. and Pratico D. (2004). Modulation of nuclear factor-kappa B activity by indomethacin influences A beta levels but not ap precursor protein metabolism in a model of Alzheimer's disease. Am. J. Pathol. 165, 2197-2206.

Tekirian T.L., Yang A.Y., Glabe C. and Geddes J.W. (1999). Toxicity of pyroglutaminated amyloid beta-peptides 3(pE)-40 and-42 is similar to that of A beta 1-40 and-42. J. Neurochem. 73, 1584-1589.

Thambisetty M., Simmons A., Velayudhan L., Hye A., Campbell J., Zhang Y., Wahlund L.O., Westman E., Kinsey A., Guntert A., Proitsi P., Powell J., Causevic M., Killick R., Lunnon K., Lynham S., Broadstock M., Choudhry F., Howlett D.R., Williams R.J., Sharp S.I., Mitchelmore C., Tunnard C., Leung R., Foy C., O'Brien D., Breen G., Furney S.J., Ward M., KLoszewska I., Mecocci P., Soininen H., Tsolaki M., Vellas B., Hodges A., Murphy D.G., Parkins S., Richardson J.C., Resnick S.M., Ferrucci L., Wong D.F., Zhou Y., Muehlboeck S., Evans A., Francis P.T., Spenger C. and Lovestone S. (2010). Association of plasma clusterin concentration with severity, pathology, and progression in Alzheimer disease. Arch. Gen. Psychiatry 67, 739-748.

Thanopoulou K., Fragkouli A., Stylianopoulou F. and Georgopoulos S.

(2010). Scavenger receptor class B type I (SR-BI) regulates perivascular macrophages and modifies amyloid pathology in an Alzheimer mouse model. Proc. Natl. Acad. Sci. USA 107, 20816-20821.

Tomiyama T., Nagata T., Shimada H., Teraoka R., Fukushima A., Kanemitsu H., Takuma H., Kuwano R., Imagawa M., Ataka S., Wada Y., Yoshioka E., Nishizaki T., Watanabe Y. and Mori H. (2008). A new amyloid mu variant favoring oligomerization in Alzheimer's-type dementia. Ann. Neurol. 63, 377-387.

Tomiyama T., Matsuyama S., Iso H., Umeda T., Takuma H., Ohnishi K., Ishibashi K., Teraoka R., Sakama N., Yamashita T., Nishitsuji K., Ito K., Shimada H., Lambert M.P., Klein W.L. and Mori H. (2010). A mouse model of amyloid beta oligomers: their contribution to synaptic alteration, abnormal tau phosphorylation, glial activation, and neuronal loss in vivo. J. Neurosci. 30, 4845-4856.

Tong Y., Xu Y., Scearce-Levie K., Ptacek L.J. and Fu Y.H. (2010). COL25A1 triggers and promotes Alzheimer's disease-like pathology in vivo. Neurogenetics 11, 41-52.

van Groen T. and Kadish I. (2005). Transgenic AD model mice, effects of potential anti-AD treatments on inflammation and pathology. Brain Res. Rev. 48, 370-378.

Van Nostrand W.E., Xu F., Rozemuller A.J. and Colton C.A. (2010). Enhanced capillary amyloid angiopathy-associated pathology in Tg-SwDI mice with deleted nitric oxide synthase 2. Stroke 41, S135-138

Vassar R., Bennett B.D., Babu-Khan S., Kahn S., Mendiaz E.A., Denis P., Teplow D.B., Ross S., Amarante P., Loeloff R., Luo Y., Fisher S., Fuller L., Edenson S., Lile J., Jarosinski M.A., Biere A.L., Curran E., Burgess T., Louis J.C., Collins F., Treanor J., Rogers G. and Citron M. (1999). beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science 286, 735-741.

Vermes E., Kirsch M., Farrokhi T., Loisance D., Fanen P. and Zimmermann R. (2004). Serum amyloid A protein: Not a relevant marker for recognition of cardiac allograft rejection. Transplantation 78, 950-951.

Vidal R., Frangione B., Rostagno A., Mead S., Revesz T., Plant G. and Ghiso J. (1999). A stop-codon mutation in the BRI gene associated with familial British dementia. Nature 399, 776-781.

Virata M.J. and Zeller R.W. (2010). Ascidians: an invertebrate chordate model to study Alzheimer's disease pathogenesis. Dis. Model Mech. 3, 377-385.

Wang C.M., Devries S., Camboni M., Glass M. and Martin P.T. (2010). Immunization with the SDPM1 peptide lowers amyloid plaque burden and improves cognitive function in the APPswePSEN1(A246E) transgenic mouse model of Alzheimer's disease. Neurobiol. Dis. 39, 409-422.

Wang H.Y., Lee D.H., D'Andrea M.R., Peterson P.A., Shank R.P. and Reitz A.B. (2000). beta-Amyloid(1-42) binds to alpha7 nicotinic acetylcholine receptor with high affinity. Implications for Alzheimer's disease pathology. J. Biol. Chem. 275, 5626-5632.

Whitehead S.N., Massoni E., Cheng G., Hachinski V.C., Cimino M., Balduini W. and Cechetto D.F. (2010). Triflusal reduces cerebral ischemia induced inflammation in a combined mouse model of Alzheimer's disease and stroke. Brain Res. 1366, 246-256.

Wilcock D.M., Alamed J., Gottschall P.E., Grimm J., Rosenthal A., Pons J., Ronan V., Symmonds K., Gordon M.N. and Morgan D. (2006). Deglycosylated anti-amyloid-beta antibodies eliminate cognitive deficits and reduce parenchymal amyloid with minimal vascular consequences in aged amyloid precursor protein transgenic mice. J. Neurosci. 26, 5340-5346.

Wilcock D.M., Lewis M.R., Van Nostrand W.E., Davis J., Previti M.L., Gharkholonarehe N., Vitek M.P. and Colton C.A. (2008). Progression of amyloid pathology to Alzheimer's disease pathology in an amyloid precursor protein transgenic mouse model by removal of nitric oxide synthase 2. J. Neurosci. 28, 1537-1545.

Wilcock D.M., Vitek M.P. and Colton C.A. (2009). Vascular amyloid alters astrocytic water and potassium channels in mouse models and humans with Alzheimer's disease. Neuroscience 159, 1055-1069.

Wirths O., Weis J., Kayed R., Saido T.C. and Bayer T.A. (2007). Age-dependent axonal degeneration in an Alzheimer mouse model. Neurobiol. Aging 28, 1689-1699.

Wirths O., Breyhan H., Cynis H., Schilling S., Demuth H.U. and Bayer T.A. (2009). Intraneuronal pyroglutamate-Aß 3-42 triggers neurodegeneration and lethal neurological deficits in a transgenic mouse model. Acta Neuropathol. 118, 487-496.

Wirths O., Bethge T., Marcello A., Harmeier A., Jawhar S., Lucassen P.J., Multhaup G., Brody D.L., Esparza T., Ingelsson M., Kalimo H., Lannfelt L. and Bayer T.A. (2010a). Pyroglutamate Aß pathology in APP/PS1KI mice, sporadic and familial Alzheimer's disease cases. J. Neural Transm. 117, 85-96.

Wirths O., Breyhan H., Marcello A., Cotel M.C., Bruck W. and Bayer T.A. (2010b). Inflammatory changes are tightly associated with neurodegeneration in the brain and spinal cord of the APP/PS1KI mouse model of Alzheimer's disease. Neurobiol. Aging 31, 747-757.

Wolfe M.S., Xia W.M., Ostaszewski B.L., Diehl T.S., Kimberly W.T. and Selkoe D.J. (1999). Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity. Nature 398, 513-517.

Wolozin B. (2004). Cholesterol and the biology of Alzheimer's disease. Neuron 41, 7-10.

Woodhouse A., Vickers J.C., Adlard P.A. and Dickson T.C. (2009). Dystrophic neurites in TgCRND8 and Tg2576 mice mimic human pathological brain aging. Neurobiol. Aging 30, 864-874.

Wu H.Y., Hudry E., Hashimoto T., Kuchibhotla K., Rozkalne A., Fan Z., Spires-Jones T., Xie H., rbel-Ornath M., Grosskreutz C.L., Bacskai B.J., Hyman B.T., Wu H.Y., Hudry E., Hashimoto T., Kuchibhotla K., Rozkalne A., Fan Z., Spires-Jones T., Xie H., rbel-Ornath M., Grosskreutz C.L., Bacskai B.J. and Hyman B.T. (2010). Amyloid beta induces the morphological neurodegenerative triad of spine loss, dendritic simplification, and neuritic dystrophies through calcineurin activation. J. Neurosci. 30, 2636-2649.

Xiong H., Callaghan D., Jones A., Walker D.G., Lue L.F., Beach T.G., Sue L.I., Woulfe J., Xu H., Stanimirovic D.B. and Zhang W. (2008). Cholesterol retention in Alzheimer's brain is responsible for high beta- and gamma-secretase activities and Aß production. Neurobiol. Dis. 29, 422-437.

Yamakawa H., Yagishita S., Futai E. and Ishiura S. (2010). beta-Secretase inhibitor potency is decreased by aberrant beta-cleavage location of the "Swedish mutant" amyloid precursor protein. J. Biol. Chem. 285, 1634-1642.

Yan R.Q., Bienkowski M.J., Shuck M.E., Miao H.Y., Tory M.C., Pauley A.M., Brashler J.R., Stratman N.C., Mathews W.R., Buhl A.E., Carter D.B., Tomasselli A.G., Parodi L.A., Heinrikson R.L. and Gurney M.E. (1999). Membrane-anchored aspartyl protease with Alzheimer's disease beta-secretase activity. Nature 402, 533-537.

Yang D.S., Stavrides P., Mohan P.S., Kaushik S., Kumar A., Ohno M., Schmidt S.D., Wesson D., Bandyopadhyay U., Jiang Y., Pawlik M.,

Peterhoff C.M., Yang A.J., Wilson D.A., St George-Hyslop P., Westaway D., Mathews P.M., Levy E., Cuervo A.M. and Nixon R.A. (2011). Reversal of autophagy dysfunction in the TgCRND8 mouse model of Alzheimer's disease ameliorates amyloid pathologies and memory deficits. Brain 134, 1-77.

Yao J., Irwin R.W., Zhao L., Nilsen J., Hamilton R.T. and Brinton R.D. (2009). Mitochondrial bioenergetic deficit precedes Alzheimer's pathology in female mouse model of Alzheimer's disease. Proc. Natl. Acad. Sci. USA 106, 14670-14675.

Yuede C.M., Zimmerman S.D., Dong H., Kling M.J., Bero A.W., Holtzman D.M., Timson B.F. and Csernansky J.G. (2009). Effects of voluntary and forced exercise on plaque deposition, hippocampal volume, and behavior in the Tg2576 mouse model of Alzheimer's disease. Neurobiol. Dis. 35, 426-432.

Zhang J.M., Wu X.B., Qin C., Qi J., Ma S.B., Zhang H.Y., Kong Q.L., Chen D.Q., Ba D.N. and He W. (2003). A novel recombinant adeno-associated virus vaccine reduces behavioral impairment and beta-amyloid plaques in a mouse model of Alzheimer's disease. Neurobiol. Dis. 14, 365-379.

Zhang X.M., Cai Y., Xiong K., Cai H., Luo X.G., Feng J.C., Clough R.W., Struble R.G., Patrylo P.R. and Yan X.X. (2009). Beta-secretase-1 elevation in transgenic mouse models of Alzheimer's disease is associated with synaptic/axonal pathology and amyloidogenesis: implications for neuritic plaque development. Eur. J. Neurosci. 30, 2271-2283.

Zhang X.M., Xiong K., Cai Y., Cai H., Luo X.G., Feng J.C., Clough R.W., Patrylo P.R., Struble R.G. and Yan X.X. (2010). Functional deprivation promotes amyloid plaque pathogenesis in Tg2576 mouse olfactory bulb and piriform cortex. Eur. J. Neurosci. 31, 710-721.

Zhao J., Fu Y., Yasvoina M., Shao P., Hitt B., O'Connor T., Logan S., Maus E., Citron M., Berry R., Binder L. and Vassar R. (2007). Beta-site amyloid precursor protein cleaving enzyme 1 levels become elevated in neurons around amyloid plaques: implications for Alzheimer's disease pathogenesis. J. Neurosci. 27, 3639-3649.

Zhiyou C., Yong Y., Shanquan S., Jun Z., Liangguo H., Ling Y. and Jieying L. (2009). Upregulation of BACE1 and beta-amyloid protein mediated by chronic cerebral hypoperfusion contributes to cognitive impairment and pathogenesis of Alzheimer's disease. Neurochem. Res. 34, 1226-1235.

Zhu Y., Hou H., Rezai-Zadeh K., Giunta B., Ruscin A., Gemma C., Jin J., Dragicevic N., Bradshaw P., Rasool S., Glabe C.G., Ehrhart J., Bickford P., Mori T., Obregon D., Town T. and Tan J. (2011). CD45 deficiency drives amyloid- peptide oligomers and neuronal loss in Alzheimer's disease mice. J. Neurosci. 31, 1355-1365.

Zhuo J.M. and Pratico D. (2010). Acceleration of brain amyloidosis in an Alzheimer's disease mouse model by a folate, vitamin B6 and B12-deficient diet. Exp. Gerontol. 45, 195-201.

Zou J.T., Yao Z.B., Zhang G., Wang H.Q., Xu J., Yew D.T. and Forster E.L. (2008). Vaccination of Alzheimer's model mice with adenovirus vector containing quadrivalent foldable A beta(1-15) reduces A beta burden and behavioral impairment without A beta-specific T cell response. J. Neurol. Sci. 272, 87-98.

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