Animal Models of Alzheimer Disease: Historical Pitfalls and a Path Forward

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Summary

Alzheimer disease (AD) is a medically and financially overwhelming condition, and incidence rates are expected to triple by 2050. Despite decades of research in animal models of AD, the disease remains incompletely understood, with few treatment options. This review summarizes historical and current AD research efforts, with emphasis on the disparity between preclinical animal studies and the reality of human disease and how this has impacted clinical trials. We provide a mechanism for shifting the focus of AD research away from animal models to focus primarily on human biology as a means to improve the applicability of research findings to human disease. Implementation of these alternatives may hasten development of improved strategies to prevent, detect, ameliorate, and possibly cure this devastating disease.

Keywords: Alzheimer disease, animal experimentation, clinical research, research models, therapy

1 Introduction

Alzheimer disease (AD), the primary cause of dementia in the middle-aged and elderly, is a debilitating, ultimately fatal disease characterized by progressive neurological deterioration. In addition to the medical severity of the disease, the annual cost of AD care worldwide exceeds $600 billion. Currently, more than 36 million people live with some form of dementia, and this number is expected to triple by 2050. In light of this frightening prospect, new and effective methods for preventing, detecting, and treating AD are desperately needed.

Over the last several decades, an abundance of research has been carried out, primarily in transgenic rodents, in an attempt to characterize the onset and course of AD. A number of candidate therapeutics has shown great promise in these animal models. Unfortunately, these studies have rarely translated into clinical benefits for patients. Today, AD remains difficult to diagnose and ultimately incurable, with few treatment strategies for disease management. This review details historical and current AD research efforts, highlighting the results of clinical trials for potential therapeutics, as well as the disparity between preclinical animal studies and the reality of human disease. We discuss current standards for diagnosis of AD, the limited options for treatment of the disease, and potential prevention strategies. Ultimately, we lay out a roadmap for shifting the focus of AD research away from animal models to focus primarily on human biology as a means to improve the applicability of research findings to human disease. Implementation of these alternatives may hasten development of improved strategies to prevent, detect, ameliorate, and possibly cure this devastating disease.

2 Epidemiological and clinical characteristics of Alzheimer disease

Dementia is a syndrome caused by central nervous system (CNS) disease and characterized by gradual decline of cognitive function (Alzheimer's Disease International, 2009; Fitzpatrick et al., 2005). Worldwide, an estimated 36.5 million people currently live with some form of dementia. This number is predicted to reach more than 65 million by 2030 and more than 115 million by 2050 (Obermeyer et al., 2012). The most common cause of dementia is Alzheimer disease (AD), representing 50-75% of dementia cases (Alzheimer's Disease International, 2009). The number of people living with AD in the United States alone is expected to increase from 5.2 million in 2013 to 13.8 million in 2050 (Alzheimer's Association, 2013; Hebert et al., 2013).

Alzheimer disease was first described by Alois Alzheimer in 1906, and is a fatal disease characterized by progressive loss of cognitive abilities (Wilkins and Brody, 1969). AD may be categorized into early and late onset. Early-onset AD typically begins between the ages of 30 and 60 years, and accounts for fewer than 5% of AD cases. The more common form, late-onset or spo-

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radic AD, presents after the age of 65 (Irvine et al., 2008; Rossor et al., 1996). Forgetfulness and difficulties with routine tasks are typically the initial symptoms. As the disease progresses, AD patients develop more severe memory loss, speech impairment, visual and spatial deficits, and loss of coordination and fine motor control (Rossor et al., 1996; Obermeyer et al., 2012; Alzheimer's Disease Internatinal, 2009). In addition to the cognitive, sensory, and motor deficits caused by the progression of AD, there are a number of behavioral and psychological symptoms related to dementia. These symptoms include agitation and aggression, wandering, disturbances in the sleep cycle, depression, anxiety, delusions and hallucinations (Ferri et al., 2004; de Vugt et al., 2005; Obermeyer et al., 2012). AD reduces the life expectancy of those affected, with an average survival time after diagnosis of 5-7 years (Fitzpatrick et al., 2005; Ganguli et al., 2005).

The single greatest risk factor for developing AD is age, with a risk of 10% for persons older than 65 years and nearly 50% for those older than 85 years (Rossor et al., 1996; Gatz et al., 2006; Chai, 2007). Onset of disease before age 65 is uncommon and suggests involvement of a genetic component. Autosomal dominant familial AD (FAD), which is typically an early onset AD, has been linked to mutations in genes encoding presenilin1 (PSEN1), presenilin2 (PSEN2), and amyloid beta (A4) precursor protein (AβPP) (Schellenberg et al., 1992; Sherrington et al., 1995; Levy-Lahad et al., 1995; Goate et al., 1991; Chartier-Harlin et al., 1991; Mullan et al., 1992). Inheritance of the apolipoprotein E type 4 allele (ApoE4) may increase the risk of developing sporadic late-onset AD, though ApoE4 alone is neither necessary nor sufficient to cause AD (Strittmatter et al., 1993; Corder et al., 1993; Sadigh-Eteghad et al., 2012; Rossor et al., 1996).

In addition to being a devastating physical illness, AD places a dramatic emotional and economic burden on caretakers and the healthcare system. According to the Alzheimer's Association, eighty percent of care provided to individuals with AD is provided by unpaid caregivers such as family members and friends (Schulz and Martire, 2004; Schneider et al., 1999; Alzheimer's Association, 2013; Prince and Dementia Research, 2004). In 2012 alone, American caregivers provided approximately 17 billion hours of unpaid care to those with AD. The time spent providing this unpaid care is valued at an estimated $216 billion (Alzheimer's Association, 2013). Additionally, more than sixty percent of AD caregivers "*rate the emotional stress of caregiving as high or very high; more than one-third report symptoms of depression*" (Alzheimer's Association, 2013; Zarit et al., 1980; Zarit et al., 1986; Cuijpers, 2005; Schulz and Beach, 1999). Due to the physical and emotional toll of caregiving, dementia caregivers in the United States had $9.1 billion in additional health care costs of their own in 2012 (Alzheimer's Association, 2013). In 2010, the worldwide cost of providing care to individuals with AD and other forms of dementia reached more than $604 billion (Obermeyer et al., 2012). The direct cost for AD care in the United States in 2013 is projected to be $203 billion, including $142 billion in Medicare- and Medicaid-related costs. With the number of people suffering from AD expected to triple by 2050, estimated cost of care is expected to reach more than $1 trillion per year in the United States (Alzheimer's Association, 2013).

3 Alzheimer disease pathology and basic science animal research

A number of genetic mutations have been associated with the pathological changes that occur in the brain during the course of AD, particularly in cases of FAD. In order to gain greater understanding of the progression of AD, researchers have taken advantage of these known mutations by developing transgenic animals – primarily mice – who express these mutated proteins. Using both transgenic and natural models, scientists hope to extrapolate information regarding the development and progression of an AD-like disease in animals to what actually occurs in humans. The following section provides a brief overview of AD pathology and some of the most commonly used models, what they were designed to accomplish, and the limitations that have restricted their contribution to human health. Specific attributes of various models will be discussed in detail later.

On a cellular level, AD is associated with the development of beta-amyloid (Aβ) plaques and neurofibrillary tangles (NFT) within and surrounding neurons of the CNS (Glenner and Wong, 1984; Wood et al., 1986; Kosik et al., 1986). Aβ, a protein normally found in the healthy brain, is derived from AβPP (Shoji et al., 1992). AβPP is cleaved first by β-secretase (BACE1) at amino acids 1 and 11 (Cai et al., 2001). A second, carboxyterminal cleavage is then performed by the γ-secretase complex (composed of PSEN 1 and 2, nicastrin, and anterior pharynx-defective 1) (Wolfe, 2006). Depending on where AβPP is cleaved by γ-secretase, one of two Aβ isoforms may be released: Aβ1-40 or Aβ1-42. Aβ1-42 is more prone to oligomerization and more readily forms the aggregates present in AD (Schroeter et al., 2003; Cai et al., 2001; Iwatsubo et al., 1994). Deposition of Aβ plaques in the brain is also associated with neuronal damage and activation of local astrocytes and microglia, resulting in localized inflammatory responses (Veerhuis, 2011; Eikelenboom and Stam, 1982; Eikelenboom et al., 1989; Eikelenboom and Veerhuis, 1996; Rosenberg, 2005). However, accumulation of Aβ plaques is not necessarily either a cause or a consequence of AD, as plaques can be found in the brains of cognitively healthy individuals (Walsh et al., 2000).

NFT result from hyperphosphorylation and accumulation of tau protein, a microtubule-associated protein known to play a role in axonal growth and plasticity (Wood et al., 1986; Kosik et al., 1986). It is important to note that neither Aβ plaques nor NFT are specific to AD, and both have been found in the brains of individuals with frontotemporal dementia (FTD), Parkinson disease, Huntington disease, Down syndrome, and amyotrophic lateral sclerosis, as well as in apparently normal brains (Masters et al., 1985; Ross and Poirier, 2004). What exactly triggers Aβ plaque and NFT formation, how plaques and NFT are related (if at all), as well as how these traits contribute to AD symptoms, remains incompletely understood.

Development of transgenic mouse models of AD pathology has been a popular research method (Tab. 1). As one of the hallmarks of AD, extracellular accumulation of β-amyloid peptides has been a common target (Hardy and Higgins, 1992; NIA, 1997). Three distinct mutations in AβPP have been associated with development of FAD, named for the places in which they

Tab. 1: Common mouse models of Alzheimer disease, relevant transgenes and mutations (if applicable), and the promoter under which transgenes are expressed

Model	Transgene(s)	Mutation(s)	Promoter	Reference
PDAPP	hAPP	V717F	PDGF	Games et al., 1995
H6	hAPP	N/A	PDGF	Wyss-Coray et al., 1997
J9	hAPP	N/A	PDGF	Chin et al., 2005
J20	hAPP	N/A	PDGF	Chin et al., 2005
Tg2576	hAPP	K670N, M671L	Hamster prion protein	Hsiao et al., 1996
APP23	hAPP	K670N, M671L	Thy-1.2	Sturchler-Pierrat et al., 1997
C3-3	Chimeric human/ murine APP	K670N, M671L	Mouse prion promoter	Borchelt et al., 2007
CRND8	hAPP	K670N, M671L, V717F	Hamster prion promoter	Chishti et al., 2001
APPDutch	hAPP	E693Q	Thy-1.2	Herzig et al., 2004
ARC6/ARC48	hAPP	E22G	PDGF	Cheng et al., 2004
C3-3 x PSEN1	Chimeric human/ murine APP, hPSEN1	APP K670N, M671L; PSEN A246E	Mouse prion promoter (APP, PSEN1)	Borchelt et al., 2007
PSAPP	hAPP, hPSEN1	APP K670N, M671L; PSEN M146L	Hamster prion protein (APP), HMG-CoA reductace (PSEN)	McGowan et al., 1999
$APP_{SL}PS1_{M146L}$	hAPP, hPSEN1	APP K670N, M671L, V717I; PSEN M146L	Thy-1 (APP), HMG-CoA reductace (PSEN)	Langui et al., 2004
$APP_{SL}PS1_{ki}$	hAPP, hPSEN1	APP M233T, L235P; PSEN1 M233T, L235P	Thy1 (APP)	Casas et al., 2004
5XFAD	hAPP, hPSEN1	APP K670N, M671L, I716V, V717I; PSEN1 M146L, L286V	Thy1	Oakley et al., 2006
hBACE1/hAPP	hAPP, hBACE1	N/A	Thy1 (BACE1, APP)	Bodendorf et al., 2002
Htau	hTau	mTauKO	Tau, Thy1	Andorfer et al., 2003
TauP301L	hTau	P301L	Thy1.2	Gotz et al., 2001
Tau V337M	hTau	V337M	PDGF	Tanemura et al., 2002
Tau P301S	hTau	P301S	Thy1.2	Allen et al., 2002
Tau G272V, P301S	hTau	G272V, P301S	Thy1.2	Schindowski et al., 2006
3xTg-AD	hAPP, hPSEN1, hTau	APP K670N, M671L; PSEN1 M146V; tau P301L	Thy1.2 (APP, Tau)	Oddo et al., 2003

were discovered: Swedish (K670N/M671L), London (V717I) and Indiana (V717F) (Duyckaerts et al., 2008; Chartier-Harlin et al., 1991; Goate et al., 1991). Numerous transgenic mouse lines have been developed which express human AβPP (hAβPP) proteins with one or more of these FAD mutations. Generally speaking, in these mice extracellular deposits of Aβ develop at various time points during the course of the animal's life. Moreover, there is evidence of inflammation in the brain, and the animals display cognitive and behavioral deficits compared to wild-type animals (Games et al., 1995; Chishti et al., 2001; Mucke et al., 2000; Sturchler-Pierrat et al., 1997; Borchelt et al., 1997; Cheng et al., 2004).

Mutations in the PSEN proteins are associated with development of early-onset AD in humans (Rogaev et al., 1995; Sherrington et al., 1995; Perez-Tur et al., 1995). Mice expressing mutated human PSEN1 (M146L or M146V) or PSEN2 alone do not develop Aβ plaques (Mattson et al., 2001; Oyama et al., 1998; Sawamura et al., 2000). However, doubly transgenic mice expressing both mutated AβPP and PSEN1 develop Aβ plaques at a much earlier age than mice expressing mutated AβPP

alone, with more severe Aβ plaque formation, neuroinflammation, and cognitive decline (Casas et al., 2004; Borchelt et al., 1997; Blanchard et al., 2003; Duff et al., 1996; Holcomb et al., 1998; McGowan et al., 1999). Expression of five genetic mutations associated with FAD (AβPP K670N/M671L (Swedish), I716V (Florida), V717I (London), PS1 M146L and L286V) in the 5XFAD mouse line results in significant acceleration of Aβ accumulation (Oakley et al., 2006). Similarly, the β-secretase BACE1 plays an important role in both homeostatic neuronal function and the pathogenesis of AD (Harrison et al., 2003). Mice expressing both BACE1 and human AβPP exhibit enhanced kinetics of Aβ formation, increased levels of Aβ peptides, and more numerous plaques than AβPP-transgenic mice (Rockenstein et al., 2005).

Use of transgenic mouse models of amyloid pathology has led to new insights regarding the processing of Aβ and the role of soluble Aβ oligomers in the pathogenesis of AD (Schaeffer et al., 2011; Morrissette et al., 2009; Cheng et al., 2007; Lesne et al., 2006). For example, in many APP transgenic mice, pathological and functional changes occur before the appearance of amyloid plaques (Schaeffer et al., 2011).

While many of these transgenic mice develop Aβ plaque pathology and cognitive impairment, they do not address the other hallmark of AD – NFT formation. To address the role of tau protein hyperphosphorylation and NFT in the pathogenesis of AD, several mouse models have been developed that overexpress either wild-type or mutated human tau protein. Notably, tau protein mutations are associated with frontotemporal dementia, but not with AD (Duyckaerts et al., 2008). Introduction of human tau proteins containing FTD mutations resulted in NFT formation (Gotz et al., 2001; Lewis et al., 2000; Tanemura et al., 2002; Allen et al., 2002). Tau protein containing both G272V and P301S mutations resulted in both NFT formation and severe cognitive deficits (Schindowski et al., 2006). In an effort to model NFT pathology which is relevant to AD rather than FTD, tau knockout mice were crossed with mice expressing human genomic tau protein, resulting in mice expressing human but not murine tau protein (htau). However, these mice express minimal NFT pathology (Andorfer et al., 2003; Duff et al., 2000; Salkovic-Petrisic et al., 2013).

In an attempt to replicate both neuroanatomical aspects of AD pathology – that is, both Aβ plaques and NFT – a triple transgenic mouse model was developed. Triple transgenic mice express human AβPP with the Swedish mutation, PSEN (M146V) and tau protein (P301L). Unlike the mouse models discussed thus far, these mice develop both Aβ plaques and NFT with tau pathology. Additionally, the mice develop other pathological and behavioral characteristics similar to AD: gliosis, synaptic damage and memory impairment (Oddo et al., 2003). These mice further support the notion that soluble Aβ oligomers are toxic, as neuronal and functional deficits appear prior to plaque or NFT formation (Morrissette et al., 2009; Schaeffer et al., 2011).

While the transgenic mice described above have been very widely used in AD research, a number of other transgenic and non-transgenic animal models have been developed as well. The senescence-accelerated mouse strains (SAMP) were developed through selective breeding rather than transgenic technology. These animals develop Aβ deposition with cognitive impairment by 6 months of age (Del Valle et al., 2010). The rat is, in general, the mostly commonly used animal in the field of neuroscience research. Rats expressing transgenes similar to those used in transgenic mouse models have been developed, though few of them have actually been shown to lead to Aβ deposition and/or NFT pathology. A transgenic rat model (TgF344-AD) was recently developed which expresses human AβPP with the Swedish mutation and PSEN (PS1ΔE9) genes, leading to development of plaques and tau pathology (Cohen et al., 2013). Targeting rat nerve growth factor receptor with a monoclonal antibody conjugated to saporin (192 IgG-saporin) results in loss of cholinergic neurons and some cognitive impairment (Wiley et al., 1991). Evidence suggesting a connection between AD and impaired brain insulin signaling led to the development of a model in which rats are treated with streptozotocin (STZ), a drug that targets insulin-producing beta cells of the pancreas for destruction (Grunblatt et al., 2004; de la Monte and Wands, 2005). Rats treated with STZ develop memory and learning deficits, progressive loss of cholinergic neurons, and neurodegeneration. There have been mixed reports regarding changes to the Aβ peptide and tau protein in STZ-treated rat brains (Grunblatt et al., 2004; Prickaerts et al., 1999; Salkovic-Petrisic et al., 2006).

Unlike mice and rats, rabbits share an identical Aβ peptide sequence with humans, though they do not spontaneously develop any AD-like disease (Johnstone et al., 1991). However, wild type rabbits fed high cholesterol diets have been shown to develop Aβ deposition, tau pathology, neuronal loss, and cognitive impairment (Sparks and Schreurs, 2003). Interestingly, the increase in levels of Aβ deposition in the brain of cholesterol-fed rabbits is dependent on the presence of trace levels of copper in the drinking water, suggesting this particular metal may play a role in progression of AD (Woodruff-Pak, 2008; Woodruff-Pak et al., 2007). For this reason, researchers have believed rabbits to be useful in testing metal chelators as potential neuroprotective treatments (Ghribi et al., 2006; Woodruff-Pak et al., 2007).

Dogs have come to be considered a useful model of AD for a number of reasons. In addition to homology between a number of canine and human AβPP processing proteins, dogs, like humans, develop intricate social lives and are excellent readers of human communication and social interaction (Johnstone et al., 1991; Hare and Tomasello, 2005). As a result, cognitive function of canines can be measured not only by level of disorientation and learning deficits, but by interaction with humans as well (Sarasa and Pesini, 2009). Moreover, aging canines have been shown to develop cognitive symptoms similar to AD in humans (Landsberg, 2005; Opii et al., 2008; Cummings et al., 1996b; Tapp et al., 2003; Head et al., 1995). The brains of aged canines have also been shown to develop Aβ plaques, though they do not develop NFT. The extent of Aβ plaque pathology correlates with some measure of cognitive impairment, though, as in humans, Aβ plaque pathology has been identified in canines without any apparent cognitive symptoms (Sarasa and Pesini, 2009). Many treatment strategies in preclinical testing have been applied to canines (Head et al., 1998, 2000; Cummings et al., 1996a; Colle et al., 2000; Hou et al., 1997).

Nonhuman primates (NHP) are long-lived species and are the genetically and physiologically closest relatives to humans. The structure and organization of the NHP brain is similar to that of humans, and the sequence of NHP AβPP is identical to that of humans. Several species of NHP develop age-related neurodegenerative conditions accompanied by cognitive deficits (Martin et al., 1994; Lemere et al., 2008; Gearing et al., 1997; Schultz et al., 2000; Bons et al., 2006). Specifically, this neurodegeneration is associated with brain atrophy, abundant amyloid plaques, and loss of cholinergic neurons (Voytko, 1998). A few species develop tau pathology in addition to amyloid plaque burden (Sarasa and Pesini, 2009). Interestingly, despite these similarities between humans and NHP, some researchers are proposing development of transgenic NHP for the purpose of studying AD (Chen et al., 2012; Chan and Yang, 2009).

4 Correlations between animal models of AD and human AD

Despite the availability of a variety of AD animal models across several species, use of these models has rarely translated into health benefits for humans with AD. This is almost certainly due to the imperfect replication of human AD in any other animal species, as well as species-specific functions of structurally identical genes and responses to targeted therapies. To date, no single animal model has exhibited both neuropathological and behavioral symptoms characterizing human AD. A variety of intrinsic differences between animal models of AD and human disease are responsible for these shortcomings (Duyckaerts et al., 2008).

Perhaps the most obvious divergence from human disease in transgenic animal models is the artificial nature of transgenic technology. First, the transgenic animals developed for AD research express human genes containing mutations associated with FAD. However, a very high percentage of AD cases are sporadic and not associated with any particular genetic mutation. As a result, the animal models that have been developed to model AD are not clearly relevant to the dominant form of AD occurring in humans (Alzheimer's Association, 2013; Chai, 2007; Alzheimer's Disease International, 2009; Rossor et al., 1996). Transgenic animals in AD research express human AD-related genes that have been artificially introduced into an embryo under control of a promoter. As a result, there is no way to control such key factors as where in the genome the transgene will insert, how many copies will be inserted, and the manner in which the inserted gene will undergo transcription, RNA processing, and protein post-translational modification (Davis et al., 2012; Geghman and Li, 2011; Toman et al., 1999). Moreover, the tissue in which the gene is expressed, as well as the timing and abundance of expression, are largely dependent on the promoter introduced with the gene (Duyckaerts et al., 2008). Many of the available transgenic mouse models utilize different promoters, contributing to the discrepancies in results among animal studies. Finally, because transgenes in rodent models of AD are foreign (i.e., human), high expression of these genes may trigger unpredictable responses or defense mechanisms in cells (Duyckaerts et al., 2008; Davis et al., 2012; Thyagarajan et al., 2003). This may confound interpretation of results from experiments utilizing AD rodent models, as it is not always clear whether a particular pathology is due to AD-like disease or some developmental abnormality resulting from the overexpression of a human gene in a rodent cell.

Beyond the artificial nature of transgenic rodent models, mice and rats developing AD-like symptoms and pathology do not faithfully recapitulate human AD in any model. Single-transgenic rodent models exhibit very little neuronal loss during the course of disease, whereas human AD is characterized by progressive loss of cholinergic neurons (Duyckaerts et al., 2008; Calhoun et al., 1998; Lewis et al., 2000). Conversely, in a number of the double – or triple – transgenic mouse models, moderate to severe neuronal loss has been observed, primarily in the hippocampus (Irizarry et al., 1997; Ishihara et al., 1999; Casas et al., 2004; Schmitz et al., 2004; Urbanc et al., 2002). However, the sheer variability in results from these different model systems makes it difficult to extrapolate data for application to human disease. Moreover, AD in humans results in significant atrophy of the brain, particularly in the entorhinal cortex, hippocampus, and amygdala (Duyckaerts et al., 2008). While brain atrophy is observed in transgenic mice, it occurs very early in life (by three months of age) and prior to accumulation of Aβ (Dodart et al., 2000; Gonzalez-Lima et al., 2001; Redwine et al., 2003; Valla et al., 2006). One possible explanation is that brain atrophy in transgenic animals is a developmental defect, unrelated to AD-like pathology (Duyckaerts et al., 2008).

Another important discrepancy between rodent and human AD pathology is the localization of Aβ plaques and NFT. In humans, Aβ deposits begin exclusively in the neocortex. Pathology then extends into allocortical brain regions, such as the hippocampus. Regions of the diencephalon, where the thalamus and hypothalamus are located, as well as the striatum and cholinergic nuclei of the basal forebrain are affected next. Finally, in the late stages of disease, Aβ pathology can be detected in regions of the brain stem and the cerebellum. The plaques that are seen in the cerebellum are "diffuse" while the plaques that are thought to be most pathogenic, and the ones specifically required for a diagnosis of AD, are "neuritic" plaques.

In mouse models of AD, however, the spatial distribution of pathology depends largely on the promoter used to drive expression of the transgenes (Duyckaerts et al., 2008; Davis et al., 2012). It is worth noting that even with ubiquitous neuronal expression in some mouse models (e.g., tg2576) there is still a remarkable regional specificity, with plaques primarily in hippocampus and cortex. As discussed previously, many of the commonly used models of AD utilize a variety of different promoters, resulting in variation in pathological topography. While canine Aβ pathology appears largely in the cortex and hippocampus (similar to humans), the brain stem, cerebellum, entorhinal cortex, amygdala, basal ganglia and olfactory bulbs are largely unaffected (Hou et al., 1997; Head et al., 2000). Aged rhesus macaques exhibit loss of cholinergic neurons, much like humans affected by AD, but the cortex of the macaque brain remains largely unaffected by AD-like pathology (Rapp and Amaral, 1992; Siddiqi and Peters, 1999).

While differences in the relative distribution of Aβ deposits throughout the brain are cause for concern when considering animal models, another important distinction between human disease and animal models is the physical nature of the Aβ peptide itself. Murine Aβ aggregates demonstrate immunoreactivity with human Aβ-specific antibodies and stain similarly with thioflavin-S and Congo red (Duyckaerts et al., 2008). However, there are a number of significant differences between human and animal Aβ plaques. In humans, Aβ peptides are the primary components of so-called senile plaques (Roher et al., 1993; Sergeant et al., 2003). Multiple different isoforms of Aβ are present in these plaques, including $A\beta_{1-40}$, $A\beta_{1-42}$, and numerous N-truncated peptides (Sergeant et al., 2003). Moreover, these peptides undergo a number of post-translational modifications, such as isomerization, racemization, pyroglutamyl formation, oxidation, and covalent linkage of Aβ dimers (Kuo et al., 2001). The cumulative effect of these modifications results in a peptide that is largely insoluble. In mice, Aβ deposits are compact and laminar, and the murine Aβ peptide is completely soluble in solutions containing the denaturing agent sodium dodecyl sulfate (Kuo et al., 2001; Kalback et al., 2002). This is likely due to the lack of post-translational modification of Aβ in the mouse model (Kuo et al., 2001). As Kuo et al. note, "*It is possible that the processing required to create authentic AD plaques cannot occur in transgenic animals because either the necessary enzyme homologs are not present or the elevated pace of amyloid deposition simply precludes the prerequisite maturational reactions*," thus underscoring another serious limitation of transgenic animal models (Kuo et al., 2001).

In the canine model of AD, Aβ deposits tend to be diffuse and fibrillar, which only resembles early stages of AD in the human brain (Torp et al., 2000; Cotman and Head, 2008). Despite their close phylogenetic relationship to humans, NHP exhibit significant differences in Aβ plaque characteristics as well. In aged macaques, vascular Aβ deposits stain strongly against $A\beta_{1-42}$, while both $A\beta_{1-40}$ and $A\beta_{1-42}$ are present in human senile plaques (Nakamura et al., 1995; Kanemaru et al., 1996). Additionally, aged macaques possess a significantly greater ratio of $A\beta_{1-40}$ to $A\beta_{1-42}$ than human patients with AD, suggesting that NHP may undergo fundamental differences in amyloid processing (Gearing et al., 1994, 1996).

As previously discussed, formation of Aβ-containing plaques represents only a portion of the pathological changes that occur during the course of AD. Hyperphosphorylation of tau protein, leading to the development of NFT, is a second important hallmark of AD. To date, no transgenic mouse model, and only a single rat model, has been shown to develop both Aβ plaques and NFT. Several of the Aβ transgenic mouse lines develop hyperphosphorylation of tau protein, but they do not develop NFT (Blanchard et al., 1997; Lazarov et al., 2007; Masliah et al., 2001; Shukkur et al., 2006). This underscores both the variability among transgenic models and the incomplete representation of human disease in these animals. Transgenic mice expressing normal human tau protein isoforms exhibit hyperphosphorylation of tau protein, but not NFT formation (Brion et al., 1999; Duff et al., 2000; Gotz et al., 1995; Ishihara et al., 1999; Probst et al., 2000; Spittaels et al., 1999). One exception to this is a transgenic mouse line than expresses human tau protein, but not mouse tau protein, resulting in NFT formation and implying that mouse tau protein prevents formation of NFT (Andorfer et al., 2003). This is likely due to expression of different tau protein isoforms in each species (Duyckaerts et al., 2008). NFT formation has been observed in several other transgenic mouse models in which a mutated human tau protein has been introduced (Gotz et al., 2001; Allen et al., 2002; Lewis et al., 2000; Tanemura et al., 2002; Schindowski et al., 2006). It is important to note that mutations in tau protein are not associated with human AD (Duyckaerts et al., 2008; Spillantini et al., 1998). Additionally, expression of mutated tau protein has led to formation of NFT in spinal motor neurons in mice, a phenomenon that does not occur in human AD (Lewis et al., 2000).

Despite being touted as an excellent model of human AD, canine brains tend not to exhibit tau pathology and NFT formation (Papaioannou et al., 2001; Wegiel et al., 1998; Satou et al., 1997; Pugliese et al., 2006). Tau pathology can be detected in the brains of only some species of NHP (Schultz et al., 2000; Lemere et al., 2008; Rosen et al., 2008). Importantly, when tau pathology does occur in NHP, it is found both in neurons and glial cells in various parts of the brain, an occurrence not associated with human AD (Kiatipattanasakul et al., 2000).

The presence of Aβ plaques provides stimuli to surrounding astrocytes and microglia, resulting in induction of localized immune responses in the human AD brain. Aβ is capable of activating complement and inducing expression of proinflammatory cytokines such as IL-1β, IL-6, TNF-α, and some chemokines (Eikelenboom and Veerhuis, 1996; Rubio-Perez and Morillas-Ruiz, 2012; Rosenberg, 2005; Akiyama et al., 2000). In mouse models of AD, however, there have been conflicting reports regarding the nature of the inflammatory response to Aβ. Studies by different groups using the same transgenic mouse line have demonstrated different profiles of proinflammatory cytokine expression in the region of plaques (Benzing et al., 1999; Matsuoka et al., 2001; Mehlhorn et al., 2000). Moreover, a direct comparison of inflammation in transgenic mouse brains and brains from AD patients revealed an overall less profound inflammatory response in the mouse model (Schwab et al., 2004).

Finally, it is essential to note that animal models of AD are chosen or designed primarily on the basis of cognitive decline and AD-like pathology (specifically, the presence of plaques and/or tangles). However, the exact cause of neuronal dysfunction and death resulting in cognitive decline remains unclear. In human AD, the abundance of fibrillar Aβ plaques correlates poorly with the severity of dementia, though NFT burden may correlate to disease progression (Dickson et al., 1995; Terry et al., 1991; Bierer et al., 1995). Rather, recent data suggest that soluble Aβ oligomers, which precede extracellular deposition of the protein and plaque formation, are responsible for synaptic dysfunction in the AD brain (Walsh and Selkoe, 2007). Even in mouse models, it has been reported that cognitive decline is evident prior to deposition of Aβ plaques in the CNS (Westerman et al., 2002; Wu et al., 2004; Moechars et al., 1999; Hsia et al., 1999; Chapman et al., 1999). It is likely, then, that detection and treatment of AD prior to deposition of plaques and presentation of cognitive

symptoms is necessary. However, because we do not understand the earliest stages of AD as it occurs in the human brain, there is no way to accurately model it in an animal such that it will readily translate into therapeutic benefit in patients.

It is clear that there are fundamental differences between human AD and the pathology present in animal models. Among these differences are characteristics of Aβ peptide itself and plaque formation, concurrent presence of Aβ and NFT, gross localization of pathology, and properties of the resulting immune response. Variation in the principal pathology of AD, combined with the lack of clarity regarding what actually causes AD, render animal models problematic for uncovering disease mechanisms and new therapeutics for the benefit of human health.

5 Preclinical drug development research for Alzheimer disease

A great deal of preclinical drug development research has been performed, or is ongoing, to shed light on AD risk factors and prevention, and to develop improved therapeutics. Numerous animal models have been used to develop diverse potential therapeutics targeting multiple different aspects of AD pathology. The following section briefly summarizes several of the different categories of potential therapeutics, their intended targets, and how they have fared in preclinical animal tests.

One of several neurodegenerative pathologies associated with AD is the loss of cholinergic neurons. These neurons produce the neurotransmitter acetylcholine, the loss of which is associated with development of memory deficits in AD. Acetylcholinesterase, the enzyme that hydrolyzes acetylcholine, has been observed to co-localize with Aβ aggregates in the brain and to enhance the aggregation of Aβ *in vitro* (Inestrosa et al., 1996). Inhibition of acetylcholinesterase has become an attractive drug target, and a number of candidate drugs have been tested in AD transgenic mice. Donepezil significantly enhanced cognitive performance in mice expressing mutated human AβPP (Van Dam et al., 2008). Donepezil, as well as galantamine and tacrine, other acetylcholinesterase inhibitors, improved short-term memory and attention deficits induced by scopolamine in rats (Kirkby et al., 1996; Bores et al., 1996). Additionally, galantamine reversed cognitive deficits in mice who had received lesions to a region of the brain involved in memory (Sweeney et al., 1988; Sweeney et al., 1989). Ispronicline improved cognition and memory in mice with a favorable safety profile in rodents and dogs (Lippiello et al., 2006; Gatto et al., 2004). Finally, phenserine has been shown to significantly improve cognition in both rodent and canine models of AD (Haroutunian et al., 1997; Klein, 2007; Lilja et al., 2013a,b; Patel et al., 1998).

Modulation of nicotinic and muscarinic receptors can modify acetylcholine metabolism as well. M1 muscarinic receptor agonists talsaclidine, AF-102B and AF-267B (NGX-267) improved cognitive outcomes in mouse, rabbit and lemur models of AD. These drugs reduced Aβ-related oxidative stress and apoptosis in neurons and decreased tau protein hyperphosphorylation (Fisher et al., 2002a,b). Similarly, the nicotinic receptor agonist ABT-089 has resulted in improvements in cognitive function in both rats and NHP (Sullivan et al., 1997; Decker et al., 1997; Rueter et al., 2004).

Memantine, a N-methyl-D-aspartate (NMDA) receptor antagonist, improved cognitive ability in mouse models of AD. NMDA receptors are involved in synaptic plasticity and synapse formation, which play important roles in memory and learning (Minkeviciene et al., 2004; Mayer and Westbrook, 1987). In several studies involving cognitive impairment in rats, memantine reversed memory and learning deficits (Zajaczkowski et al., 1996; Wenk et al., 1997; Barnes et al., 1996). On a cellular level, rat neurons were protected from Aβ-related apoptosis and neurodegeneration when treated with memantine (Miguel-Hidalgo et al., 2002). These findings are consistent with evidence that hyperactivation of NMDA receptors may contribute to neuronal injury in AD (Danysz and Parsons, 2003).

The AChEI drug tacrine, when produced as the dimer bis(7)-tacrine, reduced generation of Aβ by blocking NMDA receptors as well as directly inhibiting the β-secretase involved in Aβ processing in *in vitro* studies (Fu et al., 2008). Treatment with bis(7)-tacrine also reduced the severity of memory deficit in rat models of cognitive impairment (Liu et al., 2000). Moreover, bis(7)-tacrine is able to protect neurons from Aβ-related apoptosis by modulating calcium channel activity (Fu et al., 2006). Two drugs used to treat type 2 diabetes, rosiglitazone and pioglitazone, inhibit β-secretase activity indirectly by activating nuclear peroxisome proliferator-activated receptor γ (PPARγ). Both of these drugs improved cognitive and behavioral deficits in mouse models of AD (Mangialasche et al., 2010; Heneka et al., 2005; Pedersen et al., 2006). Pursuing β-secretase in the development of therapeutics has been complicated by the multitude of non-target substrates affected by the enzyme. Inhibition of β-secretase may cause toxicity due to inadvertent inhibition of proteins such as neuregulin-1, which is involved in myelination of neurons (Mangialasche et al., 2010).

Many studies have focused on developing inhibitors of γ-secretase, also involved in processing AβPP to Aβ. Transgenic mice treated with N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT) exhibited reduced Aβ in the brain within three hours (Dovey et al., 2001). Further studies with DAPT in a different transgenic mouse model illustrated inconsistencies in cognitive outcome, with the drug reducing Aβ levels in young mice and having no effect in older mice (Lanz et al., 2003). In mice and rats, semagacestat treatment lowered plasma, cerebrospinal fluid, and brain Aβ levels (Henley et al., 2009). Much like the search for β-secretase inhibiting drugs, toxicity issues have plagued the development of successfully animal-tested γ-secretase inhibitors. Drugs targeting γ-secretase tend to cross-react with the Notch-1 protein, which plays an essential role in cell-fate determination (Geling et al., 2002; Micchelli et al., 2003). As a result, Notch-sparing γ-secretase inhibitors have been an attractive area of study. Begacestat lowered Aβ levels and improved cognitive function in AD transgenic mice without causing Notch-related toxicity (Mayer et al., 2008).

Many *in vitro* and *in vivo* studies have demonstrated associations between neurotoxicity and Aβ aggregates (Cavallucci et al., 2012). A number of mechanisms have been proposed to prevent Aβ aggregation, or to disrupt established aggregates.

Tramiprosate is a glycosaminoglycan (GAG) mimetic designed to fit into the GAG binding site of soluble Aβ, preventing aggregation (Aisen, 2005; Gervais et al., 2001). Treatment of AD transgenic mice with tramiprosate resulted in a significant reduction in Aβ plaque burden (Gervais et al., 2007). MPACs bind strongly to copper and zinc, inhibiting metal-induced Aβ aggregation and reactive oxygen species generation *in vitro* (Cherny et al., 2001). PBT2 treatment of AD transgenic mice decreased soluble brain Aβ within hours and improved cognitive performance within several days (Adlard et al., 2008). Scyllo-inositol also binds to Aβ and is thought to regulate its folding and thereby prevent aggregation. In transgenic mice, scyllo-inositol reduced concentrations of soluble and aggregated Aβ, plaque burden, synaptic loss, and local inflammatory responses, and improved cognitive function (McLaurin et al., 2006).

A number of immunization strategies, both active and passive, have been proposed to prevent or remove Aβ deposits from the brain. Researchers have developed a number of peptides with which to immunize patients against Aβ and/or Aβ aggregates, postulating that antibody opsonization of the aggregates will dissolve the plaques or lead to clearance by activated microglia (Mangialasche et al., 2010). In mice, vaccination with the Aβ peptide AN-1972 induced production of antibodies that prevent or remove Aβ aggregates (Schenk et al., 1999; Mangialasche et al., 2010). Immunization with Cad106, a short peptide mimicking a portion of the $A\beta_{1-6}$ sequence coupled to a virus-like particle, resulted in an antibody that reduced Aβ plaque in both mice and rhesus macaques (Wiessner et al., 2011). AFFITOPE technology has been utilized to develop other short (6 amino acid) peptides mimicking the N-terminus of Aβ without actually sharing its sequence for use in vaccines. Immunization of two different transgenic mouse lines with AFFITOPE AD-01 and AD-02 resulted in reduced Aβ plaque burden and improved cognitive function (Schneeberger et al., 2009).

A number of candidate monoclonal antibody formulations, such as bapineuzumab, solanezumab and ponezumab, have been developed for passive immunization. In preclinical studies, transgenic mice were treated with monoclonal antibodies raised against Aβ. Vaccinated animals exhibited reduction in Aβ aggregates and synaptic pathology. Moreover, vaccinated mice experienced fewer cognitive deficits when compared with unvaccinated controls (Morgan et al., 2000; Bard et al., 2000; Buttini et al., 2005; DeMattos et al., 2001; Dodart et al., 2002).

A variety of other classes of drugs have been developed that target amelioration of AD. Latrepirdine (Dimebon) is an antihistamine that was shown to improve learning in a rat model of AD (Lermontova et al., 2000; Bachurin et al., 2001). Both lithium and valproate, used as mood stabilizers in the treatment of bipolar disorder, showed promising effects on Aβ plaque burden in AD transgenic mice (Su et al., 2004). Metrifonate, an AChEI previously used to treat schistosomiasis, showed promising increases of acetylcholine levels in rat brains, suggesting it may be successful as a treatment for AD (Mori et al., 1995).

6 Clinical therapeutic trials for Alzheimer disease

Despite the promising outcomes of many preclinical safety and efficacy studies, the overwhelming majority of clinical trials for potential AD treatments have failed (Tab. 2). Despite the more than 1200 clinical trials related to AD listed on http://ClinicalTrials.gov, only five drugs are currently approved for treatment of AD. These drugs treat the symptoms of AD with variable effectiveness and without slowing progression of the disease (Mangialasche et al., 2010). Of the five available drugs, four are cholinesterase inhibitors. Tacrine was the first drug approved by the FDA in 1993 for the treatment of AD, though it is seldom prescribed today, as its side effects are more severe than those associated with other AChEIs. Galantamine and rivastigmine improve cognitive abilities in some patients with mild to moderate AD (Raskind et al., 2000; Wilcock et al., 2000; Kavanagh et al., 2011; Rosler et al., 1999; Farlow et al., 2000). The AChEI donepezil and the NMDA receptor antagonist memantine are the only drugs with demonstrated effectiveness in moderate to severe AD (AlzForum, 2014; Rogers and Friedhoff, 1996; Reisberg et al., 2003). Unfortunately, only about 50% of patients derive benefit from any of these drugs, and for those patients effectiveness typically is lost in six to twelve months (AlzForum, 2014).

Tab. 2: Preclinical and clinical testing results of candidate AD therapeutics as of March 2014

Drug	Target	Preclinical results	Clinical trial results
Donepezil	Cholinesterase	Enhanced cognitive performance in mice, improved short-term memory and attention deficits induced by scopolamine in rats (Dawson and Iversen, 1993)	FDA approved for all disease stages (AlzForum, 2014)
Galantamine	Cholinesterase	Improved short-term memory and attention deficits induced by scopolamine in rats, reversed cognitive deficits in mice who had received CNS lesions (Sweeney et al., 1988, 1989; Chopin and Briley, 1992)	FDA approved for mild to moderate AD (AlzForum, 2014)
Rivastigmine	Cholinesterase	Ameliorated aging-induced learning deficits and cholinergic dysfunction in rats (Fisher et al., 2003)	FDA approved for mild to moderate AD (AlzForum, 2014)
Tacrine	Cholinesterase	Improved short-term memory and attention deficits induced by scopolamine in rats (Chopin and Briley, 1992)	FDA approved for mild to moderate AD (AlzForum, 2014)
Phenserine	Cholinesterase	Significantly improved cognition in both rodents and canines (Haroutunian et al, 1997; Patel et al., 1998; Klein, 2007)	Not effective (Becker and Greig, 2012)

Drug	Target	Preclinical results	Clinical trial results
Ispronicline	Cholinesterase	Enhanced memory in mice and rats (Gatto et al., 2004; Lippiello et al., 2006)	Not effective (Frolich et al., 2011)
ABT-089	Cholinesterase	Improved baseline and impaired cognitive function in rats (Decker et al., 1997)	Terminated (NIH, 2011)
Latrepirdine	Cholinesterase	Improved AD pathology and learning in mice and rats (Lermontova et al., 2000; Perez et al., 2012; Steele and Gandy, 2013)	Not effective (Neale, 2010)
Metrifonate	Cholinesterase	Improved acetylcholine levels in rats (Mori et al., 1995)	Withdrawn from development (Lopez-Arrieta and Schneider, 2006)
EVP-6124	Cholinesterase	Restored memory function in rats (Prickaerts et al., 2012)	In clinical trials (Hilt et al., 2012)
Talsaclidine	M1 muscarinic receptor	Improved cognitive outcome in mouse, rabbit, and lemur models of AD (Leusch et al., 2000)	Undesirable side effects (Wienrich et al., 2001)
AF-102B	M1 muscarinic receptor	Improved cognitive outcome in mouse, rabbit, and lemur models of AD (Fisher et al., 2003)	Undesirable side effects (AlzForum, 2014)
AF267B	M1 muscarinic receptor	Improved cognitive outcome in mouse, rabbit, and lemur models of AD (Fisher et al., 2003; Fisher et al., 2002a)	Undesirable side effects (AlzForum, 2014)
Memantine	NMDA receptor	Improved cognitive ability in mice (Martinez-Coria et al., 2010)	FDA approved for moderate to severe AD (Marder, 2004)
Bis(7)-tacrine	NMDA receptor	Reduced the severity of memory deficit in rats (Liu et al., 2000)	N/A
Etazolate	α-secretase	Improved cognitive function in rats (Vellas et al., 2011)	In clinical trials (Vellas et al., 2011)
Rosiglitazone	β-secretase	Improved cognitive and behavioral deficits in mice (Pedersen et al., 2006)	Not effective (Gold et al., 2010)
Pioglitazone	β-secretase	Improved cognitive and behavioral deficits in mice (Heneka et al., 2005)	Conflicting results (Miller et al., 2011)
LY2886721	β-secretase	Reduced Aβ load in mice (May et al., 2012)	Trial halted: toxicity (Eli Lilly, 2013b)
DAPT	γ-secretase	Reduced brain Aβ load within 3 h in mice (Comery et al., 2005; Portelius etal., 2009)	N/A
Semagacestat	γ-secretase	Lowered plasma, cerebrospinal fluid and brain Aβ levels in mice and rats (Henley et al., 2009)	Trial halted: enhanced cognitive decline (Eli Lilley, 2013a)
Begacestat	γ-secretase	Lowered Aβ levels and improved cognitive function in mice (Martone et al., 2009)	Not effective (Niva et al., 2013)
Tarenflurbil	γ-secretase	Improves cognitive function in mice (Kukar et al., 2007)	Not effective (AlzForum, 2014)
NIC5-15	γ-secretase	Improved memory and reduced Aβ burden in mice (AlzForum, 2014)	In clinical trials (AlzForum, 2014)
Avagacestat	γ-secretase	Ameliorated cognitive deficits in mice (Mitani et al., 2012)	Not effective (Bristol-Myers Squibb, 2012)
PBT1	Copper/ zinc	Decreased soluble brain Aβ within hours and improving cognitive performance in mice (Adlard et al., 2008)	Not effective (Sampson et al., 2012)
Tramiprosate	Soluble Aβ	Significant reduction in Aβ plaque burden in mice (Gervais et al., 2007)	Not effective (Aisen et al., 2011)
Scyllo-inositol	Aβ	Reduced concentrations of soluble and aggregated Aβ, plaque burden, synaptic loss and local inflammatory responses, and improved cognitive function in mice (Fenili et al., 2007)	Not effective (Salloway et al. 2011)
AN-1792	Aβ immunization	Production of antibodies that prevent or clear aggregation of Aβ in mice (Schenk et al., 1999)	Trial halted: aseptic meningoencephalitis (Vellas et al., 2009)
Cad106	Aβ immunization	Production of antibodies that reduced Aβ plaque in both mice and rhesus macaques (Wiessner et al., 2011)	In clinical trials (Kingwell, 2012)
AD-01	Aβ immunization	Reduced Aβ plaque burden and improved cognitive function in mice (Schneeberger et al., 2009)	In clinical trials (Panza et al., 2014)
AD-02	Aβ immunization	Reduced Aβ plaque burden and improved cognitive function in mice (Schneeberger et al., 2009)	In clinical trials (Panza et al., 2014)

Drug	Target	Preclinical results	Clinical trial results
Bapineuzumab	Aβ antibody	Reduction in Aβ aggregates and synaptic pathology, fewer cognitive deficits in mice (Bard et al., 2012)	Not effective (Salloway et al., 2009)
Solanezumab	Aβ antibody	Reduction in Aβ aggregates and synaptic pathology, fewer cognitive deficits in mice (Imbimbo et al., 2012)	Not effective (Doody et al., 2014)
Ponezumab	Aβ antibody	Reduction in Aβ aggregates and synaptic pathology, fewer cognitive deficits in mice (La Porte et al., 2012)	Not effective (AlzForum, 2014)
Gammagard	Aβ antibody	Reduced Aβ deposition, modulated inflammatory response in mice (Sudduth et al., 2013)	Not effective (AlzForum, 2014)
MABT-5102A	Aβ antibody	Reduced Aβ load and inflammation in mice (Adolfsson et al., 2012)	In clinical trials (NIH, 2013c)

Among AChEIs, phenserine, ispronicline and ABT-089 have been tested in patients with AD, but resulted in no significant change in cognitive function (Klein, 2007; Becker and Greig, 2012; NIH, 2011). Talsaclidine, AF-102B, and AF-267B resulted in undesirable side effects, such as increased salivation, and are no longer being developed for treatment of AD (Mangialasche et al., 2010; Hock et al., 2003; Heinrich et al., 2009). Similarly, the β-secretase inhibitors LY2886721 and rosiglitazone, and γ-secretase inhibitors avagacestat and tarenflurbil entered clinical trials and resulted in either no improvement in cognitive function in AD patients or, in the case of LY2886721, toxicity to those receiving the drug (Green et al., 2009; Imbimbo and Giardina, 2011; Bristol-Myers Squibb, 2012; Eli Lilly and Company, 2013b). Semagacestat development was halted when it led to worsening of AD symptoms (Eli Lilly and Company, 2013a; Samson, 2010; Extance, 2010). Drugs designed to prevent or eliminate aggregation of Aβ, tramiprosate and scyllo-inositol, produced no improvement in cognitive function. Scyllo-inositol is currently being studied as a possible treatment for aggression in AD patients (Aisen et al., 2011; Salloway et al., 2011; NIH, 2013a). MPAC PBT1 failed to improve cognitive outcome in patients with AD and was withdrawn from development, though PBT2 remains in clinical trials (Sampson et al., 2012).

Recently, promising results from animal studies of active or passive immunotherapy for AD have garnered enthusiasm for subsequent clinical trials. Clinical trials with AN-1792, the first active immunization against Aβ, were halted when a number of patients developed aseptic meningoencephalitis (Orgogozo et al., 2003). Trials with passive immunotherapy candidates bapineuzumab, PF-04360365, and IVIg (Gammagard), have failed to slow disease progression or improve cognition in AD patients (Baxter InternationalInc, 2013; Salloway et al., 2009; Dodel et al., 2013; Pfizer, 2011). Solanezumab was not effective in patients with moderately severe AD, and its effect on mild AD is currently being studied (Tayeb et al., 2013; Imbimbo et al., 2012).

Despite the high failure rate for clinical trials of AD drugs and the limited benefits of approved drugs, a number of candidate therapeutics remain in the pipeline. EVP-6124, a nicotinic receptor agonist, showed sufficient cognitive benefits in a six-month phase II trial of 409 subjects to be advanced to a phase III trial (Hilt, 2012). NIC5-15, a Notch-sparing γ-secretase inhibitor, was well tolerated in phase I trials and is currently undergoing phase II testing (Pharmeceuticals, 2006). The α-secretase activator etazolate was well-tolerated in phase I trials. A phase II trial with etazolate showed promising outcomes on AD patient cognition, and the drug is under further development (Vellas et al., 2011). Green tea extract epigallocatechin gallate (ECGC), also an inhibitor of Aβ aggregation, recently entered phase II/III trials (Zhang et al., 2013a; Smid et al., 2012; NIH, 2013b).

Although development of immunotherapy strategies to treat AD has been disappointing, two candidate therapeutics are currently in phase II trials. AD02, a vaccine against Aβ, was safe and stabilized cognition in AD patients (NIH, 2012, 2010). MABT-5102A, a humanized monoclonal antibody directed against Aβ, exhibited a favorable safety profile in phase I testing (Adolfsson et al., 2012; NIH, 2013c). However, the consistent history of late-stage failures of AD drugs suggests that promising phase I and II trials are not predictive for phase III success. Failures have been related both to inefficacy and toxicities, emphasizing the limitations in predictability of animal models for AD drug testing, and adding to the evidence that AD drug development and testing should be reformed.

7 Modifiable lifestyle factors in AD

While the development of pharmaceuticals is an important venture, other areas of research have focused on the effects of nutritional supplements and modifiable lifestyle factors on AD. Identifying nutritional factors and lifestyle modifications that prevent, delay, or attenuate AD symptoms would be beneficial to patients, as these approaches are inexpensive and typically associated with beneficial rather than adverse side effects. Over the course of the last decade, researchers have begun to explore modifiable risk factors with prevention in mind, and a number of studies in this area have yielded promising results.

Much of the AD prevention data currently available suggest a link between cardiovascular disease and the development of AD or other dementias (Mayo Clinic, 2013; Honjo et al., 2012; Duke Medical News, 2013; Roberts et al., 2013). A study of adults possessing at least one ApoE4 allele revealed that hypertensive individuals tended to have significantly higher Aβ burdens than those with normal blood pressure (Rodrigue et al., 2013). Moreover, cholesterol is involved in generation and deposition of Aβ in the brain, though cholesterol modification

has not yet been shown to ameliorate AD symptoms (Puglielli et al., 2003; Roberts et al., 2013; Feldman et al., 2010). Lifestyle factors such as dietary composition and physical activity, which have a positive effect on cardiovascular health, may also aid in preventing AD (Roberts et al., 2013; Mayo Clinic, 2013).

The Chicago Health and Aging Project (CHAP) is "*a longitudinal population study of common chronic health problems of older persons, especially of risk factors for incident Alzheimer's disease, in a biracial neighborhood of the south side of Chicago*"(Bienias et al., 2003). This study has identified saturated fat and trans-unsaturated fat as positive correlates with development of AD, while omega-6 polyunsaturated fat and monounsaturated fat were associated with a decreased risk of AD (Morris et al., 2003). A similar study in New York revealed comparable correlations between high intake of calories, cholesterol, and saturated fat and the development of dementia (Luchsinger et al., 2002). Although treatment of AD with statins has not been successful, use of statins earlier in life is associated with decreased risk of AD, suggesting that potential benefits of statins may be limited to prevention (Feldman et al., 2010; Cramer et al., 2008; Haag et al., 2009). Similarly, a Finnish study revealed a link between high serum cholesterol and elevated risk of AD later in life, suggesting again that lower cholesterol may decrease AD risk (Notkola et al., 1998).

Long-chain omega-3 fatty acids, a type of polyunsaturated fat, may play a role in prevention of AD. Docosahexaenoic acid (DHA) is the principal omega-3 fatty acid component of neuronal membranes (Cunnane et al., 2012). DHA is especially abundant in areas of the brain such as the cerebral cortex, synaptosomes, and mitochondria. Neurons do not possess the enzymes necessary for *de novo* synthesis of DHA, so it must either be obtained directly from the diet or synthesized endogenously from its precursors α-linolenic acid and eicosapentaenoic acid (Cunnane et al., 2012; Salem et al., 1996). In a number of epidemiological studies in the US and Europe, consumption of omega-3 fatty acids and fish at least once a week was associated with decreased incidences of AD and general cognitive decline (Morris et al., 2003; Schaefer et al., 2006; van Gelder et al., 2007; Barberger-Gateau et al., 2007).

The antioxidant properties of vitamins E and C are of interest in the AD field, as oxidative stress in CNS neurons disrupts normal cellular function and contributes to AD pathology (Morris et al., 2002; Gutteridge, 1994; Simonian and Coyle, 1996; Smith and Perry, 1995; Sopher et al., 1996). A number of studies have been carried out regarding the effect of vitamins E and C on preventing AD, but with conflicting results. In CHAP and the Rotterdam Study, vitamin E intake was associated with decreased risk of AD (Morris et al., 2002; Engelhart et al., 2002). Results from the Rotterdam study also suggest a role for vitamin C in AD prevention (Engelhart et al., 2002). However, a study regarding both vitamin E and C intake in New York revealed no correlation between antioxidant supplementation and decreased incidence of AD, and other studies investigating the effects of omega-3 fatty acids and antioxidants on established AD have shown no benefit (Freund-Levi et al., 2009; Luchsinger et al., 2003; Isaac et al., 2008).

The link between cardiovascular health and AD risk extends beyond dietary factors to incorporate physical activity levels as well. A number of promising studies report that physical activity slows cognitive decline in the elderly, with or without a diagnosis of AD (Weuve et al., 2004; Pitkala et al., 2013). Moreover, individuals who engage in physical activity at mid-life appear to be less susceptible to AD (Rovio et al., 2005; Podewils et al., 2005; Scarmeas et al., 2009; Larson et al., 2006; Middleton et al., 2008; de Bruijn et al., 2013). While the mechanisms underlying the effect of exercise on cognition require more study, there is evidence to suggest that physical activity improves brain plasticity, and may lead to increases in prefrontal and hippocampal volume (Erickson et al., 2012; Radak et al., 2010). The association between exercise and reduced risk of AD may be more pronounced in individuals carrying the ApoE4 allele, though this association requires more study (Rovio et al., 2005; Podewils et al., 2005; Pizzie et al., 2013). Promisingly, a recent study suggested that exercise may improve cognitive ability and semantic memory retrieval in patients already diagnosed with mild cognitive impairment (Smith et al., 2013).

Overall, a strong argument can be made for the role of diet and physical activity in AD prevention. Altogether, the results of the cited studies suggest a diet of foods such as nuts, cruciferous vegetables, fruit, and dark, leafy vegetables may put individuals at a lower risk for developing AD later in life. Avoiding foods high in saturated and trans-unsaturated fats, such as red meat, butter, and dairy products will contribute to a lower risk of AD. Moreover, regular physical activity may independently lower the risk of developing dementia (Wilbur et al., 2012).

8 The path forward: humanizing AD research

Although animal models used to investigate AD have largely failed to yield mechanistic or treatment insights that conclusively correlate with human AD pathology or predict the safety and efficacy of therapeutic drugs, gaining an understanding of the mechanisms underlying AD pathology remains essential. In this review, we have detailed the ways in which current animal models of AD fail to accurately reflect the disease as it occurs in humans. Rather than continuing to use existing models or attempting to create new transgenic lines, evidence indicates that we should move away from animal models. Researchers should take advantage of postmortem studies, human patient samples, and tissue biobanks. Moreover, advances in induced pluripotent stem cell technology show great promise for neurodegenerative disease research (Almeida et al., 2013; Boissart et al., 2013; Burkhardt et al., 2013; Ieda, 2013; Zhang et al., 2013b). These basic science resources, combined with epidemiological studies, observational research with AD patients, and clinical trials, will allow researchers to have access to results that are directly relevant to AD in the species of interest.

Biomarker validation has become a hot research topic in the AD field. Transcriptomics, such as DNA/mRNA microarrays, has led to identification of a few genes whose expression is altered in AD, but this technology alone has not proven sensitive enough for broad biomarker detection (Humpel, 2011).

Recently, there has been enthusiasm for a combined genetic and proteomic approach (Thambisetty and Lovestone, 2010; Humpel, 2011). The ideal biomarkers will be stable, present in easily accessible patient samples (blood, CSF), with notable differences between age-matched healthy controls and individuals with AD (Humpel, 2011; Verwey et al., 2009; Lewczuk et al., 2008). Standardized diagnostics will increase the accuracy with which biomarker validation is performed.

Ideally, research funds will be directed toward validating biomarkers and optimizing rapid, accurate biomarker detection methods. Development of patient-specific DNA, RNA and proteomic chips able to detect multiple biomarkers simultaneously is of great interest (Humpel, 2011). Expanded use of Pittsburgh Compound Blue positron emission tomography to detect and track amyloid deposition in the brain may enhance the search for viable biomarkers of AD. Recent research indicates that spatial distribution of amyloid deposits, rather than total amyloid burden, correlates well with cognitive decline (Yotter et al., 2013). Use of this in combination with cognitive outcome will allow researchers to more accurately track the progression of mild cognitive impairment to AD. Despite the availability of blood and CSF samples from patients, some researchers insist on validating biomarkers in the aforementioned animal models (Rose et al., 2012; Spencer et al., 2013; Tai et al., 2013). Restricting biomarker research to human samples may produce viable candidates while eliminating unreliable interpretation of data that may occur when translating results from animals to humans. Together, these approaches will enhance the ability of researchers to evaluate the efficacy of preventive strategies or biomarker correlates.

Finally, reinforcing clinical trial education for both patients and physicians may increase the number of individuals willing to enroll in clinical trials. In recent years, pharmaceutical and device companies have moved many operations overseas (Garnier, 2008). Among NIH-funded studies, insufficient domestic enrollment has forced investigators to seek participants internationally (Kim et al., 2011; Eapen et al., 2013). While this is, to some degree, the result of the expense and complex regulations governing clinical studies in the US, physician involvement in clinical studies plays a role as well. Results from a Research American poll indicated that 75% of potential participants surveyed would be interested in participating in a clinical trial (Charlton Research Company, 2009). Medical school and residency training programs can be enhanced by requiring physicians in training to learn both why and how to enroll patients in clinical trials (Eapen et al., 2013). Moreover, outreach and education to the American public regarding the importance of clinical trial participation and what trials may be available to them will only serve to improve the quality of US trials and their applicability to US patients (Eapen et al., 2013).

9 Conclusions

The U.S. Department of Health and Human Services recently updated the *National Plan to Address Alzheimer's Disease*. This update calls for increased enrollment in clinical trials and expanded research to identify biomarkers, genetic risk factors, and pharmaceutical and lifestyle interventions. The inclusion of studies on the roles of lifestyle factors in the prevention or treatment of AD is laudable. However, despite making lifestyle intervention research a priority, only a small fraction of NIH research dollars allocated to AD studies go to researchers studying nutrition, physical activity, or models of dementia care. With the overwhelming failure of drug trials in recent years, it is imperative that we shift our focus and cash flow to these prevention and intervention studies, affording them the same importance we place on biomarker and risk factor research. Moreover, increasing our investment in basic science research that takes advantage of human-based technologies, such as inducible pluripotent stem cells, improves the likelihood of discoveries that are applicable to human disease.

Alzheimer disease exacts a physically, emotionally, and financially devastating toll on those affected by it. With the incidence of AD expected to triple in the next several decades, it is more important than ever that preventive measures and successful treatments be established. Current basic research strategies involving animal models of AD have been unable to achieve these goals due to unreliability and poor translation to human AD. By removing the emphasis of AD research from animal models and placing it on the human patient, we can expect to increase the likelihood of identifying methods by which AD may be prevented, ameliorated, and reversed.

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