

SPECIAL ISSUE  
REVIEW

## Molecular mechanisms for Alzheimer's disease: implications for neuroimaging and therapeutics

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### Abstract

Alzheimer's disease is a progressive neurodegenerative disorder characterised by the gradual onset of dementia. The pathological hallmarks of the disease are  $\beta$ -amyloid (A $\beta$ ) plaques, neurofibrillary tangles, synaptic loss and reactive gliosis. The current therapeutic effort is directed towards developing drugs that reduce A $\beta$  burden or toxicity by inhibiting secretase cleavage, A $\beta$  aggregation, A $\beta$  toxicity, A $\beta$  metal interactions or by promoting A $\beta$  clearance. A number of clinical trials are currently in progress based on these different therapeutic strategies and they should indicate which, if any, of these approaches will be efficacious. Current diagnosis of Alzheimer's disease is made by clinical, neuropsychologic and neuroimaging assessments. Routine structural neuroimaging evaluation with computed tomography and magnetic resonance imaging is based on non-specific features such as atrophy, a late feature in the progression of the disease,

hence the crucial importance of developing new approaches for early and specific recognition at the prodromal stages of Alzheimer's disease. Functional neuroimaging techniques such as functional magnetic resonance imaging, magnetic resonance spectroscopy, positron emission tomography and single photon emission computed tomography, possibly in conjunction with other related A $\beta$  biomarkers in plasma and CSF, could prove to be valuable in the differential diagnosis of Alzheimer's disease, as well as in assessing prognosis. With the advent of new therapeutic strategies there is increasing interest in the development of magnetic resonance imaging contrast agents and positron emission tomography and single photon emission computed tomography radioligands that will permit the assessment of A $\beta$  burden *in vivo*.

**Keywords:** Alzheimer's disease, amyloid  $\beta$ -peptide, neurodegenerative disorders, brain imaging  
*J. Neurochem.* (2006) **97**, 1700–1725.

Alzheimer's disease, the leading cause of dementia in the elderly, is an irreversible, progressive neurodegenerative disorder clinically characterised by memory loss and cognitive decline, leading invariably to death, usually within 7–10 years after diagnosis.

Age is the dominant risk factor in Alzheimer's disease. The progressive nature of neurodegeneration suggests an age-dependent process that ultimately leads to synaptic failure and neuronal damage (Masters and Beyreuther 1998) in cortical areas of the brain essential for memory and higher mental functions. The increase in the number of new cases of Alzheimer's disease is the direct consequence of an improvement in life expectancy. Despite the tremendous

corpus of knowledge of genetics, epidemiology, risk factors and neuropathological mechanisms, there is still no cure for Alzheimer's disease.

Received April 20, 2006; revised manuscript received May 3, 2006; accepted May 3, 2006.

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**Abbreviations used:** A $\beta$ ,  $\beta$ -amyloid; APP, amyloid precursor protein; FDG, fluorodeoxyglucose; MPAC, metal-protein attenuated compounds; NFT, neurofibrillary tangles; PS, presenilin; ROS, reactive oxygen species.

### Clinical features and diagnostic criteria

At present, clinical diagnosis of Alzheimer's disease is based on progressive impairment of memory and decline in at least one other cognitive domain, and exclusion of other diseases that might also present with dementia, such as frontotemporal dementia, dementia with Lewy-bodies, stroke, brain tumour, normal pressure hydrocephalus or depression.

The clinical diagnostic accuracy for Alzheimer's disease depends on the stage of disease and can exceed 90% in academic settings in mid or late stages. Diagnostic criteria for Alzheimer's disease have been proposed within both the DSM and ICD classification systems. However, the criteria followed in most research studies are those proposed by the National Institute of Neurological and Communicative Disorders and Stroke – Alzheimer's Disease and Related Disorders Association (NINCDS-ARDA) for Defining Probable Alzheimer's disease (McKhann *et al.* 1984).

A variable period of up to 5 years of prodromal decline in cognition characterised by a relatively isolated impairment in long-term memory that may also be accompanied by impairments of working memory, known as mild cognitive impairment, usually precedes the formal diagnosis of Alzheimer's disease (Petersen *et al.* 1999). These deficits presumably relate to damage to the medial temporal lobe and/or specific prefrontal-temporal lobe circuits. About 40–60% of carefully characterised subjects with mild cognitive impairment will subsequently progress to meet criteria for Alzheimer's disease over a 3–4-year period (Petersen *et al.* 1999).

### Neuropathology of Alzheimer's disease

In the absence of biologic markers, direct pathologic examination of brain tissue derived from either biopsy or autopsy remains the only definitive method for establishing a diagnosis of Alzheimer's disease (Selkoe 2001; Masters and Beyreuther 2005). The typical macroscopic picture is gross cortical atrophy. Microscopically, there is widespread cellular degeneration and neuronal loss that affects primarily the outer three layers of the cerebral cortex, initially affecting more the temporal and frontal cortical regions subserving cognition than the parietal and occipital cortices. These changes are accompanied by reactive gliosis, diffuse synaptic and neuronal loss, and by the presence of the pathological hallmarks of the disease, intracellular neurofibrillary tangles (neurofibrillary tangles) and extracellular amyloid plaques (Selkoe 2001; Masters and Beyreuther 2005).

Neurofibrillary tangles are intraneuronal bundles of paired helical filaments. The main structural component of neurofibrillary tangles is a normal constituent of cellular microtubules but when present in Alzheimer's disease is an abnormally phosphorylated form, known as tau protein. They are most easily identified in the hippocampus. Neurofibrillary

tangles are not specific to Alzheimer's disease, and are found in a variety of other neurodegenerative conditions such as frontotemporal dementia, subacute sclerosing panencephalitis, Hallervorden-Spatz disease, Parkinson dementia complex and dementia pugilistica (Perl 2000). Tau is a widely expressed phosphoprotein from the microtubule associated family, the main function of which is to maintain microtubule stability. In Alzheimer's disease, hyperphosphorylated tau aggregates, reducing its ability to bind microtubules and leading to cytoskeletal degeneration and neuronal death (Lovestone and Reynolds 1997).

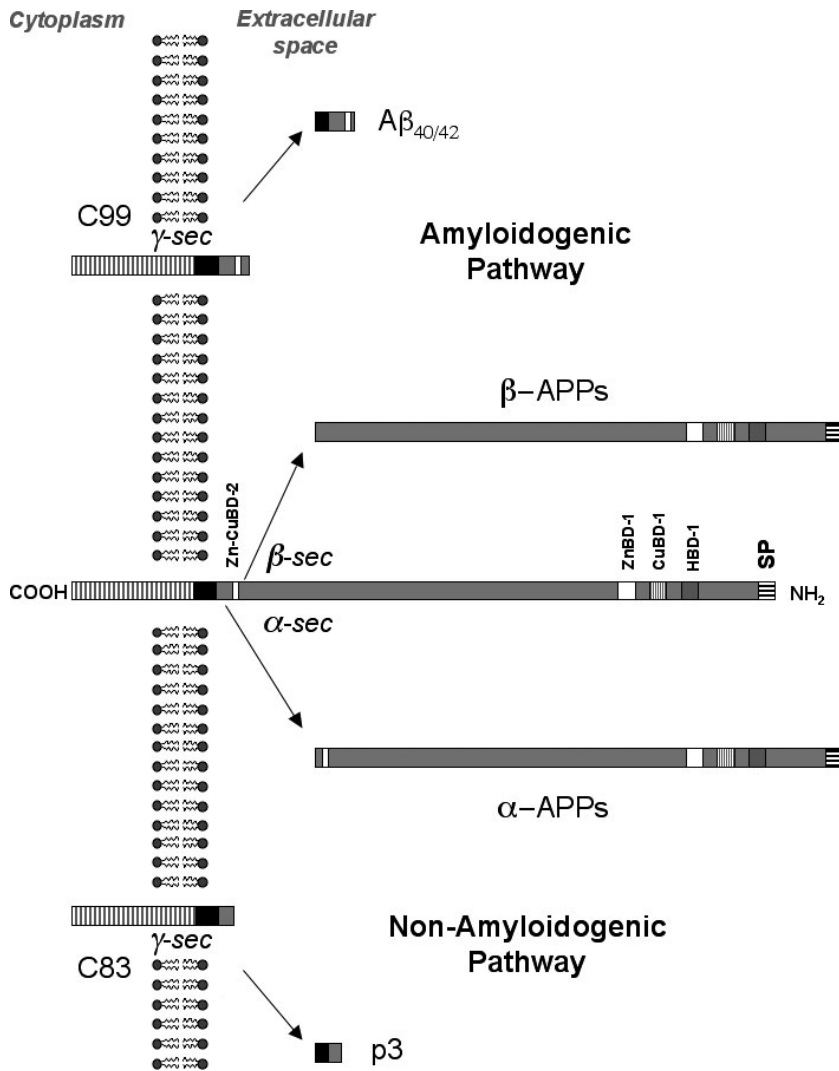
The plaques consist of extracellular aggregates of amyloid  $\beta$ -peptide ( $A\beta$ ).  $A\beta$  is a 4-kDa self-aggregating, 39–43-amino acid metalloprotein product derived from the proteolytic cleavage of the amyloid precursor protein by  $\beta$ - and  $\gamma$ -secretases (Cappai and White 1999; Selkoe 2001) (Fig. 1). The plaques are intimately surrounded by dystrophic axons and dendrites, reactive astrocytes and activated microglia (Masters and Beyreuther 2005).  $A\beta$  is not only found within senile plaques, but is also present around cortical arterioles as a congophilic angiopathy. It can also be assessed in CSF, plasma and even neuronal cultures (Seubert *et al.* 1992). A number of *in vitro* and *in vivo* studies have shown  $A\beta$  protein to be directly toxic to neurons, leading to the aggregation and secondary phosphorylation of the tau protein.

$A\beta$  was first identified and sequenced from meningeal blood vessels of Alzheimer's disease and Down's syndrome patients 20 years ago (Glennner and Wong 1984; Masters *et al.* 1985). The aggregation process that converts soluble  $A\beta$  into amyloid fibrils is thought to be a nucleation-dependent process (Harper and Lansbury 1997) requiring structural transitions of  $A\beta$ .

On electron microscopy, amyloid fibrils are composed of multiple protofibrils wrapped around each other, forming a crossed  $\beta$ -pleated sheet.

### $A\beta$ centric theory of Alzheimer's disease

Through the years, several theories have been postulated to explain the molecular mechanisms leading to Alzheimer's disease (Masters and Beyreuther 1998; Selkoe 2001). The  $A\beta$  centric theory is the dominant etiologic paradigm at this time, because it is the only one that best or most comprehensively articulates the current available knowledge regarding the cellular, molecular and functional alterations observed in Alzheimer's disease. Not only is there a wealth of histopathological, biochemical, genetic and animal model data that support the key role of  $A\beta$  in the pathogenesis of Alzheimer's disease, but no alternative hypothesis has emerged in the last two decades of intensive Alzheimer's disease research. The  $A\beta$  centric theory states that an imbalance between the production and removal of  $A\beta$  leads to its progressive accumulation, triggering a series of reactions leading to synaptic dysfunction, microgliosis and



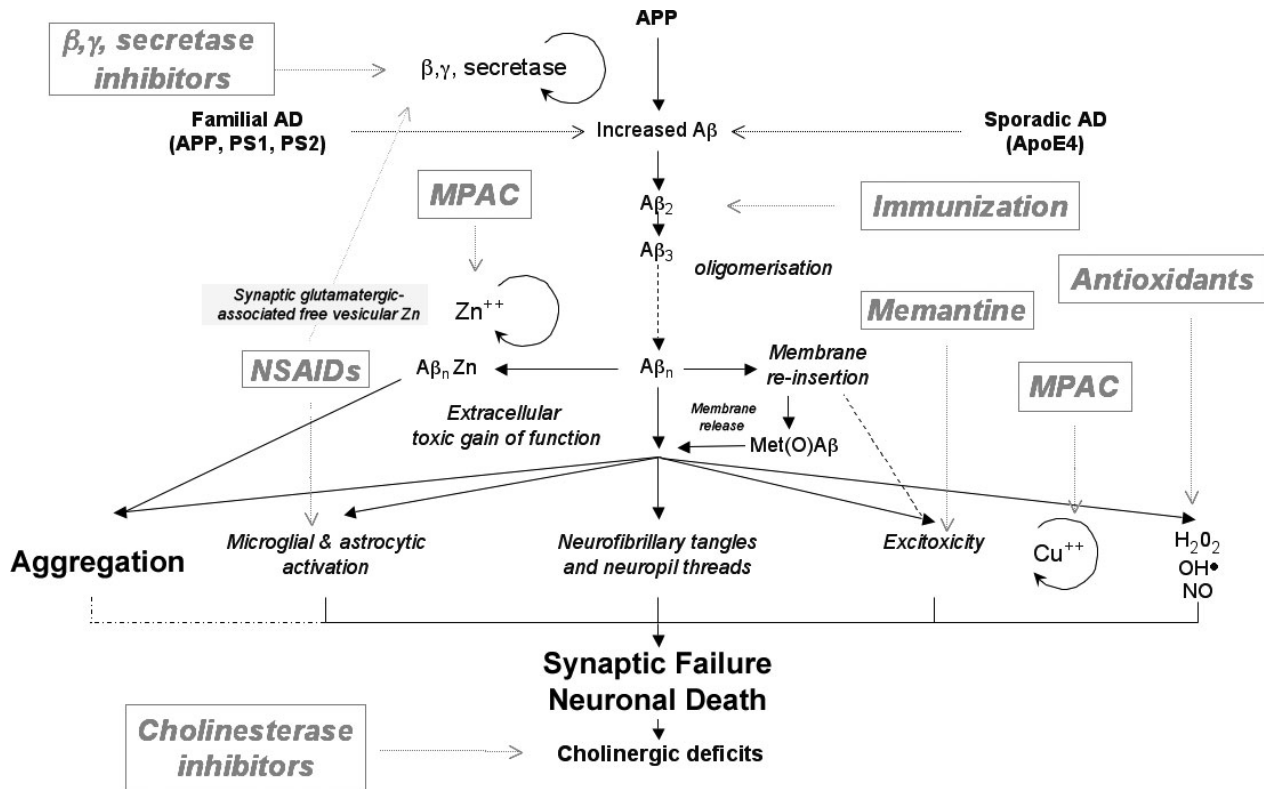
**Fig. 1** Schematic diagram of amyloidogenic and nonamyloidogenic proteolytic pathways of amyloid precursor protein and production of A $\beta$ . Amyloid precursor protein is cleaved by either  $\alpha$ -secretase ( $\alpha$ -sec) or  $\beta$ -secretase ( $\beta$ -sec) yielding  $\alpha$ -amyloid precursor proteins or  $\beta$ -amyloid precursor proteins, respectively. The C-terminal C83 fragment produced by  $\alpha$ -secretase, and the C-terminal C99 fragment produced by  $\beta$ -secretase, are then further cleaved by  $\gamma$ -secretase ( $\gamma$ -sec) into P3 or A $\beta_{40/42}$ , respectively. APP, amyloid precursor proteins; SP, signal peptide; HBD-1, heparin binding domain-1; CuBD-1, copper binding domain-1; ZnBD-1, zinc binding domain-1; Zn-CuBD-2, zinc copper binding domain-2; NH<sub>2</sub>, N-terminal end; COOH: C-terminal end.

neuronal loss, clinically manifested with memory loss and impaired cognitive functions (Selkoe 2001) (Fig. 2). The loss of synaptic function seems to be the critical factor in cognitive decline (Selkoe 2002). Much of the controversy derives from the use of the term amyloid. The broad term can be applied not only to A $\beta$ , but to several unrelated extracellular deposits of fibrillar protein, such as  $\beta_2$ -microglobulin, amylin or serum amyloid A, each one of them associated with a specific disease.

The earliest structural, microscopically visible pathological changes in Alzheimer's disease are diffuse A $\beta$  deposits. These deposits are also observed in normal ageing individuals, but the density is lower than in Alzheimer's disease patients (Perry *et al.* 1978) indicative of an immature or not yet toxic form of A $\beta$ . The presence of extracellular A $\beta$  in highly specialised cortical brain regions implicated in memory and cognition precede the other pathognomonic pathological features of Alzheimer's disease, indicating that increases in A $\beta$  are involved in the

early presymptomatic stages of the disease. As the earliest phenotypical marker of disease, this has crucial implications for neuroimaging and treatment. The increase in A $\beta$  deposition is accompanied by decreases in A $\beta$  in CSF (Sunderland *et al.* 2003).

Though extracellular amyloid plaques are the hallmark brain lesions of sporadic Alzheimer's disease, the distribution and density of both diffuse and A $\beta$  plaques at the light microscopic level (McLean *et al.* 1999) have not been consistently shown to correlate with the degree of cognitive impairment. The best correlation occurs with soluble levels of A $\beta$ , measured biochemically (McLean *et al.* 1999). Soluble A $\beta$  is in equilibrium with insoluble A $\beta$  in the plaques. The significance of the aggregated amyloid plaques can be interpreted as they either are a reservoir for the soluble oligomers, or represent the sequestered pool of soluble and now precipitated A $\beta$ , therefore fulfilling a 'protective' function, or just the end stage or final product of the A $\beta$  cascade.



**Fig. 2** Schematics showing the role of A $\beta$  in Alzheimer's disease pathogenesis along with traditional and novel therapeutic strategies. Increased production or reduced clearance of A $\beta$  leads to aggregation, deposition and neuronal injury through a variety of neurotoxic mechanisms, such as generation of oxygen and nitrogen radicals (H<sub>2</sub>O<sub>2</sub>, OH $\cdot$ , NO), transition metal ion interactions, excitotoxicity, tau hyper-

phosphorylation into neurofibrillary tangles, inflammatory response via microglia and astrocytic activation leading to synaptic deficits and cell death. The therapeutic interventions are bolded in grey and boxed and the dotted arrows indicate the target(s). AD, Alzheimer's disease; APP, amyloid precursor proteins; MPAC, metal-protein attenuating compound.

One of the criticisms raised against the amyloid hypothesis has come from some of the interpretations of the work of Braak and Braak (1991), who stated that neurofibrillary degeneration of cell bodies and their neurites not only predate morphologically detectable amyloid plaques but that they also increase gradually with age. However, as Hardy and Selkoe (2002) point out, the postmortem cases used to establish the Braak Stage I neuropathology criteria were nondemented older individuals, in whom it is impossible to distinguish whether their neurofibrillary changes represent early stages of Alzheimer's disease or a different process altogether (Price and Morris 1999). It has been well established in patients with Down's syndrome that A $\beta$  deposition predates the formation of neurofibrillary tangles (Lemere *et al.* 1996).

The A $\beta$  theory is strongly supported by compelling genetic data (Hardy and Selkoe 2002). Though it is highly probable that additional genes are associated with Alzheimer's disease, to date only three different genes, all associated with A $\beta$  production, are implicated in the pathophysiology of Alzheimer's disease, and have been

described in patients with the rare early onset familial Alzheimer's disease (St George-Hyslop 2000), mutations of the amyloid precursor protein gene (Citron *et al.* 1992; Rovelet-Lecrux *et al.* 2006) on chromosome 21, mutations in the presenilin 1 and 2 genes on chromosome 14 and 1, respectively (Rogaev *et al.* 1995; Miklossy *et al.* 2003). Presenilin 1, presenilin 2 and amyloid precursor protein have a clear-cut autosomal dominant pattern with a penetrance above 85%. Moreover, a polymorphism in the apolipoprotein E gene on chromosome 19 (Strittmatter *et al.* 1993) is the most prevalent of these risk factors for Alzheimer's disease and acts as a weaker susceptibility factor. The main feature of the mutations in amyloid precursor protein, presenilin 1 and 2 is their involvement in the different steps of amyloid precursor protein processing pathway, which leads to increased production and elevated plasma levels of A $\beta$ , specially A $\beta$ <sub>42</sub> (Scheuner *et al.* 1996). These various genetic mutations, all manifesting as a similar clinical entity and all leading to increased levels of A $\beta$ , and A $\beta$  build-up in the brain before Alzheimer's disease symptoms arise, further support the A $\beta$  theory of Alzheimer's disease.

### Amyloid precursor protein

The amyloid precursor protein gene was cloned following the purification and sequencing of the A $\beta$  peptide from Alzheimer's disease brains and is localised to chromosome 21 (Kang *et al.* 1987), the chromosome involved in Down's syndrome, a condition that invariably develops the typical Alzheimer's disease neuropathology by age 50, though they start developing amyloid plaques as early as age 12, long before they get neurofibrillary tangles and other Alzheimer's disease lesions (Lemere *et al.* 1996).

Amyloid precursor protein is a 695–770-residue ubiquitously expressed glycosylated transmembrane protein with a large hydrophilic aminoterminal extracellular domain, a single hydrophobic transmembrane domain consisting of 23 residues and a small carboxy-terminal cytoplasmic domain (Kang *et al.* 1987). Structural studies support the model that the extracellular domain is composed of a multidomain structure of seven defined regions. The N-terminal cysteine-rich domain is composed of two domains, the growth factor domain (Rossjohn *et al.* 1999) and the copper binding domain (Multhaup *et al.* 1996; Barnham *et al.* 2003a). The growth factor domain and copper binding domains are joined by a short hinge sequence. The crystal structure of the growth factor domain has a large positively charged electrostatic patch on the surface that would allow heparin binding, consistent with its heparin binding activity (Small *et al.* 1994). The NMR structure of the copper binding domain showed the copper binding site is surface exposed, unlike other copper binding proteins. It has homology to copper chaperones, in agreement with amyloid precursor protein being a modulator of copper homeostasis (White *et al.* 1999). The cysteine-rich region is followed by an acidic region and no tertiary structural data has been reported. The alternatively spliced Kunitz-protease inhibitor and OX-2 domains follow the acidic domain. The Kunitz-protease inhibitor domain has high sequence and structural homology to other Kunitz inhibitor proteins (Hynes *et al.* 1990). *In vitro* and *in vivo* studies have indicated amyloid precursor protein can modulate hemostasis processes in both a Kunitz-protease inhibitor and a non-Kunitz-protease inhibitor manner (Mahdi *et al.* 1995; Henry *et al.* 1998; Xu *et al.* 2006). The role of the OX-2 domain remains unclear. The carbohydrate domain follows either the acidic domain in the APP695 isoform or the Kunitz-protease inhibitor domain in APP751 or OX-2 domain in APP770, respectively. The carbohydrate domain undergoes both N- and O-glycosylation (Weidemann *et al.* 1989) and appears to be composed of at least two separate regions. The most proximal domain has been termed CAPPD (Dulubova *et al.* 2004), E2 (Wang and Ha 2004) or D6a (Andersen *et al.* 2006). This is a structured region composed of tightly packed helices that can interact to form an antiparallel dimer (Wang and Ha 2004). The CAPPD/E2/D6a sequence is highly conserved amongst the amyloid

precursor protein-family orthologues and paralogues, suggesting an important function. The D6a domain binds to the neuronal sorting protein sorLA and the interaction modulates amyloid precursor protein processing into A $\beta$  (Scherzer *et al.* 2004; Andersen *et al.* 2005). The distal portion of the carbohydrate domain, which contains the  $\alpha$ - and  $\beta$ -secretase cleavage sites, appears to be unstructured and is susceptible to proteolytic degradation (Dulubova *et al.* 2004). The amyloid precursor protein molecule is tethered to the membrane via a single transmembrane domain which contains the hydrophobic C-terminal portion of the A $\beta$  peptide. The cytoplasmic domain is short (51aa) and undergoes phosphorylation and binds to a number of adaptor molecules, including Fe65, X11 and Numb (Kerr and Small 2005). The cytoplasmic domain is released following  $\gamma$ -secretase cleavage and may be transported to the nucleus (Kerr and Small 2005).

The majority of amyloid precursor protein is degraded in the endoplasmic reticulum and only a small fraction enters the secretase cleavage pathway (Selkoe 2001). While amyloid precursor protein is usually proteolytically cleaved by  $\beta$ -secretase, mutations on the amyloid precursor protein gene were shown to be associated with increased A $\beta$  self-aggregation, and A $\beta$  production by the sequential cleavage by  $\beta$ - and  $\gamma$ -secretases (Citron *et al.* 1992).

The free N-terminus of A $\beta$ , considered the first critical step in amyloid formation (Selkoe 2001), is derived from the amyloid precursor protein by proteolytic cleavage by  $\beta$ -secretase. Several lines of evidence demonstrate that  $\beta$ -secretase cleavage of amyloid precursor protein is required for A $\beta$  generation (Seubert *et al.* 1993). Generation of the N-terminus is followed by C-terminal cleavage by  $\gamma$ -secretase to release the final A $\beta$ -product from the  $\beta$ -secretase cleavage fragment C99. Cleavage by  $\gamma$ -secretase occurs within the transmembrane region of amyloid precursor protein yielding mainly 40- and 42-amino acid A $\beta$  C-terminal variants, A $\beta$ 40 and A $\beta$ 42 (Fig. 1).

Amyloid precursor protein can also undergo nonamyloidogenic processing by  $\alpha$ -secretase, which cleaves amyloid precursor protein within the A $\beta$  domain to generate  $\alpha$ -amyloid precursor proteins (the ectodomain of amyloid precursor protein ending at the  $\alpha$ -secretase cleavage site) (Mudher and Lovestone 2002) and C83 (the C-terminal tail of amyloid precursor protein), which can then undergo  $\gamma$ -secretase cleavage leading to the release of p3 (Fig. 1), a shortened, probably non-pathogenic, form of A $\beta$  (Scheuner *et al.* 1996).

Although the function of amyloid precursor protein is unknown, a significant body of evidence suggests it functions in maintaining cellular Cu homeostasis (Barnham *et al.* 2004a) by possibly delivering Cu and maybe Fe to metallo-enzymes and proteins, such as superoxide dismutase 1, and the Cu ATPase. Amyloid precursor protein knockout mice have increased Cu levels in both brain and liver (White *et al.*

1999), whilst overexpression of the A $\beta$  containing carboxyl-terminal fragment of amyloid precursor protein in transgenic mouse models results in significantly reduced brain Cu not Fe levels (Maynard *et al.* 2002). Cu can modulate *in vivo* amyloid precursor protein processing (Bayer *et al.* 2003; Phinney *et al.* 2003) with higher Cu levels resulting in a reduction in A $\beta$  production and a consequential increase in the nonamyloidogenic p3 form of the peptide (Borchardt *et al.* 1999). Independent Cu-binding sites have been identified on both A $\beta$  and the amyloid precursor protein ectodomain. The Cu-binding domain of amyloid precursor protein shows some structural homology to copper chaperones. It contains a tetrahedral binding site consisting of two histidine residues (positions 147, 151), a tyrosine (position 168) and methionine (position 170) that favours Cu(I) coordination (Barnham *et al.* 2003a). The A $\beta$  Cu-binding site is located near the N-terminal part of the peptide and consists of histidines 6, 13, 14 and an as yet unidentified fourth ligand (Curtain *et al.* 2001; Smith *et al.* 2006).

### Presenilins

There is significant genetic evidence coming from mutations in the presenilin 1 and presenilin 2 genes (Rogaev *et al.* 1995; Miklossy *et al.* 2003) that the presenilin proteins affect  $\gamma$ -secretase activity (De Strooper *et al.* 1998). The majority of early onset familial Alzheimer's disease cases are linked to mutations within the presenilin genes. More than 40 mutations have been described in the gene for presenilin 1 that can subsequently result in Alzheimer's disease. Mutations in both genes selectively increase the production of A $\beta$ 42 in cultured cells and in the brains of transgenic mice and are associated with early onset familial Alzheimer's disease (Selkoe 2001). Some presenilin mutations associated with increases in A $\beta$  metabolism instead of presenting Alzheimer's disease symptoms show large plaques and special symptoms such as spastic paraparesis (Smith *et al.* 2001). Analysis of a range of presenilin 1 familial Alzheimer's disease mutations on different  $\gamma$ -secretase substrates (amyloid precursor protein, Notch, N-cadherin and N-syndecan) suggested they affected presenilin-associated  $\gamma$ -secretase activity in diverse ways (Bentahir *et al.* 2006).

Presenilin 1 and 2 are ubiquitously expressed within the brain, primarily in neurons (Rogaev *et al.* 1995; Sherrington *et al.* 1995). The proteins contain multiple transmembrane domains, with an amino and carboxy terminus as well as a large hydrophilic loop located in the cytoplasm (Rogaev *et al.* 1995; Sherrington *et al.* 1995). Both proteins, the 46-kDa presenilin 1 and 55-kDa presenilin 2, share 67% amino acid identity. The exact functions associated with presenilin protein have not been fully elucidated yet. Presenilin 1 is involved in normal neurogenesis and formation of the axial skeleton, as well as in  $\gamma$ -secretase activity. Gene deletion of presenilin 1 shows that it is indispensable for the generation of A $\beta$  (De Strooper *et al.* 1998). Two transmembrane

aspartate residues in presenilin 1 are essential for A $\beta$  production, indicating that presenilin 1 is either an essential cofactor for  $\gamma$ -secretase or maybe  $\gamma$ -secretase itself (Kimberly *et al.* 2000). Presenilin 2 also contains the two transmembrane aspartate residues which appear to be critical for  $\gamma$ -secretase activity (Kimberly *et al.* 2000).

### Apolipoprotein E

Genetic variability in A $\beta$  catabolism and clearance increase the risk for late-onset Alzheimer's disease (Bertram *et al.* 2000; Olson *et al.* 2001). In contrast to the rare, early onset autosomal dominant forms, the only consistent marker for both the early onset familial and late-onset non-familial form of dementia is the polymorphism of apolipoprotein E allele on chromosome 19 (Saunders *et al.* 1993). Encoded on the long arm of chromosome 19, apolipoprotein E is a 34-kDa lipid transport protein considered the major genetic risk factor in the pathogenesis of Alzheimer's disease (Marques and Crutcher 2003). Apolipoprotein E is normally present in oligodendroglia, astrocytes and microglia. A lipid carrier protein involved in the transport of cholesterol and phospholipids, apolipoprotein E is believed to play an important role in synaptic plasticity and neuronal repair mechanisms. It protects neuronal-glia cells cultures against H<sub>2</sub>O<sub>2</sub> oxidative injury by reducing secondary glutamate excitotoxicity *in vitro* (Lee *et al.* 2004) and is both directly and indirectly involved in oxidative mechanisms in the brain (Ramassamy *et al.* 2000). Apolipoprotein E interacts directly with A $\beta$  and with amyloid precursor protein through the carboxy-terminal domain of apolipoprotein E. The association of apolipoprotein E and A $\beta$  inhibits fibril formation (Beffert and Poirier 1998) and also attenuates glial activation by A $\beta$  (Hu *et al.* 1998). Apolipoprotein E exists in three allelic variants:  $\epsilon$ 2 (8%),  $\epsilon$ 3 (77%) and  $\epsilon$ 4 (15%). The presence of the apolipoprotein E4 allele increases fourfold the risk of Alzheimer's disease and much more if the allelic variant is inherited from both parents. The  $\epsilon$ 4 allele is absent in approximately 30–40% of patients with Alzheimer's disease and present in about 30% of healthy subjects (Mayeux *et al.* 1998) as well as in patients with Down's syndrome (St George-Hyslop *et al.* 1994). In carriers of apolipoprotein E4 allele, A $\beta$  deposition responsible for the congophilic angiopathy (Kalaria 2002) could play an important role in contributing to the chronic cortical hypoperfusion typically observed in neuroimaging studies of patients with Alzheimer's disease (Villemagne *et al.* 2005a). Whereas the  $\epsilon$ 4 allele is associated with an increased risk for Alzheimer's disease, the  $\epsilon$ 2 allele is believed to represent no increased or decreased risk and the  $\epsilon$ 3 allele may even confer some protection against A $\beta$ -induced toxicity (Corder *et al.* 1993) through its anti-oxidant and membrane-stabilising properties and via complexation and internalisation of A $\beta$  through apolipoprotein E receptors (Jordan *et al.* 1998). Furthermore, apolipoprotein E is a metal chelator, and the  $\epsilon$ 4 allele variant

binds more rapidly to A $\beta$  while at the same time displaying the weakest chelator affinity (Moir *et al.* 1999).

### Transgenic mice models

Transgenic mice models of Alzheimer's disease with mutations in amyloid precursor protein and presenilin genes lead to increase production and progressive aggregation of A $\beta$ , reproducing the major features of Alzheimer's disease: A $\beta$  plaques, associated with neuronal and microglial damage (Games *et al.* 1995; Hsiao *et al.* 1996). The difference in tau sequence between mouse and humans may explain why despite the progressive A $\beta$  deposition there are no neurofibrillary tangles and very little neuronal loss (Games *et al.* 1995; Hsiao *et al.* 1996; Irizarry *et al.* 1997). Other reasons to be considered are species differences in neuronal vulnerability, the relatively short duration of exposure to A $\beta$ , and the lack of certain cytokines necessary for a full complement inflammatory response.

Mutations in tau protein leading to large tau deposits in intracellular neurofibrillary tangles are not associated with amyloid deposits, and are clinically manifested as frontotemporal dementia with parkinsonism (Hutton *et al.* 1998; Spillantini *et al.* 1998), indicating that the neurofibrillary tangles in Alzheimer's disease are secondary to A $\beta$  production (Hardy *et al.* 1998) and probably triggered by A $\beta$  (Rapoport *et al.* 2002).

Although the density of neurofibrillary tangles correlates better than A $\beta$  aggregates with the degree of dementia (Terry *et al.* 1994), in patients with the rare presenilin 1 mutations or individuals with Down's syndrome who died prematurely from other diseases, the presence of A $\beta$  (either as diffuse deposits or typical plaques) precedes the appearance of neurofibrillary tangles (Lippa *et al.* 1998). This has been established experimentally with transgenic mice overexpressing both mutant human tau and mutant human amyloid precursor protein which have the same number and structure of amyloid plaques but a significantly higher number of tau-positive neurofibrillary tangles than transgenic mice overexpressing only mutant human tau (Lewis *et al.* 2001), indicating that the mutant amyloid precursor protein and the consequent A $\beta$  production precede and promote the formation of neurofibrillary tangles (Götz *et al.* 2001). Apolipoprotein E-deficient mice crossed with amyloid precursor protein transgenic mice showed a significant reduction in A $\beta$  deposition (Bales *et al.* 1997), supporting the role played by apolipoprotein E in the metabolism of A $\beta$ .

### Mechanism of A $\beta$ toxicity

Because of its high lipid content and high oxygen consumption, the brain is particularly susceptible to oxidative stress. Several mechanisms have been proposed to explain A $\beta$  neurotoxicity: production of reactive oxygen species such as hydrogen peroxide, nitric oxide, superoxide, highly reactive

hydroxyl radicals and nitric oxide (NO), excitotoxicity with intracellular calcium accumulation, decreased membrane fluidity, energy depletion, alteration of the cytoskeleton, inflammatory processes and alteration of metal homeostasis (Hardy and Selkoe 2002; Bush 2003; Barnham *et al.* 2004a). All of these events converge into similar pathways of synaptic disruption, necrosis or apoptosis, leading to progressive loss of specific neuronal cell populations.

A common factor in the postulated mechanisms of A $\beta$  toxicity is the oligomerisation of A $\beta$ , whether as fully formed fibrils, dimers or trimers (Rohr *et al.* 1996; Walsh *et al.* 2002) or as protofibrils (Harper *et al.* 1999). Despite several attempts, the main obstacle to the full validation of the A $\beta$  theory remains the identification *in vivo* of the specific neurotoxic A $\beta$  soluble oligomer. There is an inverse relationship between amyloid burden and oxidative damage *in vivo* as assessed by 8-OH guanosine levels in Alzheimer's disease-affected tissue. Several lines of evidence demonstrate that diffusible soluble A $\beta$  oligomers, but not monomers or insoluble amyloid fibrils, are toxic to cultured neurons and responsible for the neurotoxicity and synaptic dysfunction present in Alzheimer's disease. Micro-injection into rats of culture medium containing soluble oligomers of human A $\beta$  (in the absence of monomers and amyloid fibrils) inhibits long-term potentiation in the hippocampus (Walsh *et al.* 2002). Changes are observed in young amyloid precursor protein transgenic mice before plaque formation (Hsia *et al.* 1999; Mucke *et al.* 2000), though the diversity and unstable nature of A $\beta$  intermediates, from monomers to mature fibrils, makes it difficult to identify the specific species responsible for the neurotoxic effects.

### Generation of reactive oxygen species

Extra- and intracellular production of reactive oxygen species initiates and promotes neurodegeneration in Alzheimer's disease (Schippeling *et al.* 2000). Evidence of oxidative stress in Alzheimer's disease is manifested through higher levels of oxidised proteins (Schippeling *et al.* 2000), advanced glycation (Smith *et al.* 1995), lipid peroxidation products (Ramassamy *et al.* 2001), formation of toxic species, such as peroxides, alcohols, aldehydes, ketones, cholesterol oxide – toxic to microglial cells (Chang *et al.* 1998), cholestenone (Bernheimer *et al.* 1987), altered gene expression (Allen and Tresini 2000) and damaged DNA (Dizdaroglu 1992). A $\beta$  induces lipoperoxidation of membranes and lipid peroxidation products (Mark *et al.* 1999). Lipids are modified by reactive oxygen species and there is a high correlation between lipid peroxides, anti-oxidant enzymes, amyloid plaques and neurofibrillary tangles in Alzheimer's disease brain (Lovell *et al.* 1995). Markers of oxidative DNA damage have been localised in plaques and neurofibrillary tangles (Mecocci *et al.* 1994; Good *et al.* 1996).

Several breakdown products of oxidative stress including 4-hydroxy-2,3-nonenal (HNE) (Selley *et al.* 2002) acrolein,

malondialdehyde and F2-isoprostanes have been observed in Alzheimer's disease brains when compared to age-matched controls (Arlt *et al.* 2002). HNE modifies proteins, resulting in a multitude of effects, including inhibition of neuronal glucose and glutamate transporters (Keller *et al.* 1997) and Na-K ATPases (Mark *et al.* 1995) plus activation of kinases and dysregulation of intracellular calcium signaling that ultimately induce an apoptotic cascade (Mattson and Chan 2003; Tamagno *et al.* 2003).

Catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase, indicators of cellular defence mechanisms against oxidative stress, are increased in the hippocampus and amygdala of Alzheimer's disease patients (Pappolla *et al.* 1992). DNA bases are vulnerable to oxidative stress damage involving hydroxylation (Gabbita *et al.* 1998), protein carbonylation and nitration. Reactive oxygen species-induced calcium influx, via activation of glutamate receptors, triggering an excitotoxic response leading to cell death, has also been observed in Alzheimer's disease brains (Yamamoto *et al.* 1998; Mattson and Chan 2003). Reactive oxygen species are generated when oxygen reacts with unregulated redox-active metals. Metalloproteins such as A $\beta$  in Alzheimer's disease that might abnormally present Cu or Fe for inappropriate reaction with O<sub>2</sub> are implicated in several age-dependent neurodegenerative disorders (Barnham *et al.* 2004a).

#### Generation of reactive nitrogen species

NO-induced neurotoxicity has been extensively studied. NO is synthesised by NO synthases (NOS), and the three isoforms of NOS – endothelial (eNOS), neuronal (nNOS) and inducible (iNOS) – are present in the brain (Law *et al.* 2001). NO synthesis is activated by glutamate release accompanied by excess Ca<sup>2+</sup> influx through activation of the NMDA (Parks *et al.* 2001) and AMPA receptors (Blanchard *et al.* 2004). A $\beta$  induces NO production by interacting with glial cells or by disrupting Ca<sup>2+</sup> homeostasis through NMDA receptor (Parks *et al.* 2001). NO combines with superoxide anion forming peroxynitrite, and the resultant reactive nitrogen species can induce significant oxidative stress leading to lipid peroxidation, damaged DNA, and neuronal death.

#### Activation of inflammatory processes

A $\beta$  oligomers/fibrils are toxic for cultured neurons and activate microglia. Blocking A $\beta$  oligomers/fibrils formation prevents this toxicity (Meda *et al.* 1995). Astrocytes and microglial cells are involved in the chronic inflammatory responses in Alzheimer's disease through the up-regulated expression of phospholipase A<sub>2</sub>, leading to increased arachidonic acid/prostaglandin inflammatory pathway activity by secreting interleukin-1 (Griffin *et al.* 1989) and activation of complement pathways (Rogers *et al.* 1992), and producing a variety of potentially neurotoxic compounds, including superoxides, glutamate and NO (Brown and Bal-Price 2003).

#### Altered energy metabolism

Intermediate metabolism is essential to maintain signaling activities and depends on mitochondrial function. Disturbed energy metabolism and the appearance of degenerating mitochondria in axonal terminals is an early feature of Alzheimer's disease (Byrne 2002).

Reactive oxygen species production, Ca<sup>2+</sup> uptake and mitochondrial membrane depolarisation have been linked to neuronal apoptosis (Kruman and Mattson 1999) by disrupting the normal mitochondrial functioning, through the uncoupling of oxidative phosphorylation and impairment of cellular respiration, compromising energy production (Cadenas and Davies 2000). The mitochondrial electron transport chain specifically, cytochrome C oxidase or complex IV, is altered in Alzheimer's disease (Parker *et al.* 1994), perhaps secondary to mutated and oxidatively damaged mitochondrial DNA (Mecocci *et al.* 1994). This is supported by results with cytoplasmic hybrid or cybrid cells (Swerdlow *et al.* 1997) which resemble electron transport chain defects observed in Alzheimer's disease (Parker *et al.* 1994).

#### A $\beta$ metal interactions

The evidence supports brain metal homeostasis, specially Zn and Cu, as significantly altered in Alzheimer's disease (Bush 2003). The progressive synaptic disruption and ultimately neuronal loss observed in Alzheimer's disease might be secondary to toxic oxidative stress from excessive free-radical generation favoured by redox active transition metals bound to A $\beta$  (Smith *et al.* 1997; Sayre *et al.* 2000; Barnham *et al.* 2004a). The generation of reactive oxygen species usually requires the reaction of O<sub>2</sub> with a redox metal ion such as Cu or Fe. A $\beta$  is a metalloprotein with high *in vitro* affinity for Cu (highest), and Fe and Zn (lowest) (Atwood *et al.* 1998; Bush *et al.* 2003). A $\beta$  has been shown to coordinate transition metal ions through bridging histidine residues at positions 6, 13 and 14 (Miura *et al.* 2000; Curtain *et al.* 2001; Smith *et al.* 2006), similar to the metal coordination sphere found in the active site of superoxide dismutase (Barnham *et al.* 2004a). When A $\beta$  binds Cu and Fe, extensive redox chemical reactions take place (Opazo *et al.* 2002; Barnham *et al.* 2004a). Isolated senile plaques generate reactive oxygen species in a manner dependent upon Cu and Fe (Sayre *et al.* 2000; Opazo *et al.* 2002).

Several lines of evidence point to the participation of transition metals in A $\beta$  neurotoxicity. Brain copper and iron concentrations increase with age (Takahashi *et al.* 2001; Maynard *et al.* 2002). Very high concentrations of Cu (400  $\mu$ M), Zn (1 mM) and Fe (1 mM) have been found in plaques of Alzheimer's disease-affected brains (Lovell *et al.* 1998). Genetic ablation of the zinc transporter 3 protein, required for zinc transport into synaptic vesicles reduced plaque formation in Tg2576 transgenic mice (Lee *et al.* 2002). Two methods of inducing aggregation of A $\beta$  are (i) metal induced cross-linking leading to amorphous aggregates

and fibril formation or (ii) lowering the pH (Yoshiike *et al.* 2001). Zn, Cu and Fe induce A $\beta$  aggregation *in vitro* (Huang *et al.* 1997; Atwood *et al.* 1998). A $\beta$  accumulation within the synaptic cleft, at which high concentrations of Zn (300  $\mu$ M) and Cu (30  $\mu$ M) are released during neurotransmission, would lead to high concentrations of soluble metallated A $\beta$ , thereby promoting its toxicity and perhaps explaining the synaptic loss observed in Alzheimer's disease (Lee *et al.* 2002). The high Zn concentrations also promote the aggregation of the Cu/Fe-metallated A $\beta$ , creating a reservoir of potentially toxic A $\beta$  that is in equilibrium with the soluble pool. The large polymeric deposits of misfolded proteins not only represent the end result of the aggregation process but may act as inactive reservoirs in equilibrium with the small diffusible oligomeric toxic species responsible for the neurodegenerative pathology. Paradoxically, some emerging data suggest that A $\beta$  might have a role as an anti-oxidant, a function that may wane with aging (Bush *et al.* 2003).

In a lipid membrane environment the addition of Cu or Zn to A $\beta$  can induce a conformational change from  $\beta$ -sheet to  $\alpha$ -helix, generating an allosterically ordered membrane-penetrating oligomer (Curtain *et al.* 2001). The extensive oxidative damage associated with A $\beta$  (Martins *et al.* 1986; Butterfield *et al.* 2001) may involve calcium dysregulation, caused by either the formation of membrane calcium channels (Arispe *et al.* 1993) or modulation of an existing channel (Mattson *et al.* 1993).

Reactions of Cu with A $\beta$  lead to oxidative modifications of the peptide. Among these modifications is the formation of a methionine sulfoxide at residue 35 (Met(O)A $\beta$ ) (Barnham *et al.* 2003b) and Raman spectroscopic studies have shown that Zn and Cu are co-ordinated to the histidine residues of the deposited A $\beta$  in the senile plaque and that the Met35 of A $\beta$  is oxidised (Dong *et al.* 2003). Met(O)A $\beta$  has been isolated from Alzheimer's disease amyloid brain deposits (Naslund *et al.* 1994). Like the reduced form of A $\beta$ , synthetic Met(O)A $\beta$  is neurotoxic, and this toxicity can be rescued by catalase and clioquinol, suggesting that the reduced and oxidised peptides have similar mechanisms of action.

Another potential oxidative modification is the covalent crosslinking of A $\beta$  through dityrosine moieties at residue 10, i.e. oxidative modifications to A $\beta$  generate soluble oligomeric forms of A $\beta$ . Soluble oligomeric forms of A $\beta$  have been associated with A $\beta$  toxicity and are correlated with cognitive and memory decline (McLean *et al.* 1999). Covalently crosslinked A $\beta$ , such as that generated by the formation of dityrosine moieties, would resist clearance (Barnham *et al.* 2004a).

If the Tyr10 of A $\beta$  is replaced by an alanine residue, this renders the peptide non-toxic. This modified form of A $\beta$  is still able to form oligomers, driven by non-specific hydrophobic interactions but cannot form dityrosine crosslinked oligomers, suggesting that a specific subset of A $\beta$  oligomers

is responsible for toxicity and not the general formation of oligomers *per se* (Barnham *et al.* 2004a).

How the soluble oligomers of A $\beta$  induce neurotoxicity is still the subject of ongoing research but a general theme has emerged whereby the interaction of A $\beta$  with lipid membranes is a necessary step in neurotoxicity (Kagan *et al.* 2002; Butterfield and Boyd-Kimball 2004; Kawahara 2004; Eckert *et al.* 2005; Puglielli *et al.* 2005). These interactions cause changes in membrane fluidity, resulting in depolarisation and disorder (Eckert *et al.* 2005), pore/channel formation leading to ion channel formation and disrupted calcium homeostasis (Kagan *et al.* 2002; Kawahara 2004), lipid peroxidation via membrane-associated free radical formation (Butterfield and Boyd-Kimball 2004) and cholesterol oxidation (Puglielli *et al.* 2005). We have recently shown that the toxicity of A $\beta$ /Cu complexes correlates with lipid peroxidation and that this can be inhibited by preventing the A $\beta$ /Cu interaction (Smith *et al.* 2006).

### The road to therapeutics

To date, no therapy has been shown to halt or reverse the underlying disease process and therapy remains confined to symptomatic palliative interventions (Barrow 2002).

Given the neuronal degeneration with impairment in cholinergic transmission in hippocampal and basal fore brain, areas associated with memory and cognition, as well as decreased levels of the cholinergic markers, choline acetyltransferase and acetyl cholinesterase, most treatment strategies are based in increasing intrasynaptic acetylcholine (ACh) levels (Auld *et al.* 2002). The acetyl cholinesterase inhibitors (AChE-I) tacrine, donepezil, rivastigmine and galantamine are now approved for the treatment of Alzheimer's disease (Emilien *et al.* 2000). However, subjects treated with AChE-I respond with a paradoxical increase in AChE levels and activity. This seems to negate the intended effect of the AChE-I in increasing intrasynaptic ACh levels. At the same time, clinical trials of AChE-Is and their meta-analyses continued to show favorable, albeit mild, effects on cognitive parameters, at least during the first 6–12 months of treatment. Against this background, attention has focused on identifying other possible mechanisms of action of the AChE-Is, especially on the amyloid precursor protein/A $\beta$  pathway, and have begun to ask whether these drugs might have any disease-modifying effects (Caccamo *et al.* 2006) by attenuating the effects of A $\beta$ -induced neuronal cytotoxicity (Kimura *et al.* 2005), promoting  $\alpha$ -secretase or decreasing  $\beta$ -secretase activity (Zimmermann *et al.* 2005; Caccamo *et al.* 2006), inhibiting A $\beta$  aggregation (Belluti *et al.* 2005) or inhibiting GSK 3 $\beta$  activity and tau phosphorylation (Caccamo *et al.* 2006).

The modulation of glutamatergic transmission in Alzheimer's disease has also received increasing attention with the results of the clinical trials of the non-competitive NMDA

antagonist memantine, proposed as a safe and effective symptomatic treatment of Alzheimer's disease patients (Rogawski and Wenk 2003). Assuming the toxic soluble oligomers of A $\beta$  may inhibit long-term potentiation at the presynaptic level and that A $\beta$  promotes the endocytosis of the NMDA receptor (mediated in part through  $\alpha 7$  nicotinic ACh receptor [nAChR], protein phosphatase PP2B and tyrosine phosphatase STEP (Snyder *et al.* 2005)), the findings on the beneficial behavioral effects of memantine in both A $\beta$  toxicity models (Yamada *et al.* 2005) and amyloid precursor protein transgenic mouse models (Van Dam *et al.* 2005) will require further evaluation.

Compounds with the ability to inactivate reactive oxygen species might have therapeutic potential in the treatment of Alzheimer's disease. Existing knowledge and screens of natural product libraries have thrown up a wide variety of anti-oxidants and 'neuroprotectants' which have an effect on the actions of A $\beta$  in some experimental cell culture toxicity assays (Moosmann and Behl 2002). Many of these assays are difficult to control, and there is little agreement in the field as to their validity. Though it has been proposed that cholinergic drugs are more effective in the treatment of Alzheimer's disease if used in association with anti-oxidants than the individual agents alone (Prasad *et al.* 2000), there has been limited clinical evaluation of the efficacy of anti-oxidants. Nevertheless, an increasing number of papers are appearing reporting efficacy of compounds derived from plants [ferulic acid (Sultana *et al.* 2005), green tea extracts (Rezai-Zadeh *et al.* 2005), curcumin (Yang F. *et al.* 2005), resveratrol (Marambaud *et al.* 2005) fucoidan (Jhamandas *et al.* 2005) and various other plant materials (Lecanu *et al.* 2005)], as well as other natural products [docosahexaenoic acid (Lim *et al.* 2005), vitamin E (Quintanilla *et al.* 2005), oestrogens (Quintanilla *et al.* 2005), glutathione (Woltjer *et al.* 2005), melatonin (Quinn *et al.* 2005), gelsolin (Qiao *et al.* 2005) and insulin-like growth factor 1 (Aguado-Llera *et al.* 2005)] and a variety of small compounds (Caraci *et al.* 2005; Marrazzo *et al.* 2005). The classical lipophilic free radical scavenger,  $\alpha$ -tocopherol (vitamin E) has been evaluated in both Alzheimer's disease and Parkinson's disease, and though it showed some encouraging results in Alzheimer's disease patients (Sano *et al.* 1997), especially when combined with ascorbic acid (Bano and Parihar 1997), it was found to have no beneficial effects in Parkinson's disease. Use of coenzyme Q10, L-carnitine and creatine might prevent mitochondrial oxidative damage and mitochondrial mutations (Moreira *et al.* 2005). Up-regulation of reactive oxygen species-scavenging enzyme capacities through neurotrophins may provide a mechanism for the prevention of neurotoxicity (Spina *et al.* 1992). There is a growing interest in the use of polyphenolic anti-oxidants to reverse age-related decline in neuronal signal transduction and cognitive and motor behavior deficits (Parihar and Hemnani 2003). Reactive oxygen species generation triggers glutamate-mediated

excitotoxicity. Memantine, which targets the NMDA receptor, slows the development of the disease and is of modest benefit to patients in the moderately severe to severe range of the disease (Reisberg *et al.* 2003) (Fig. 2). From these investigations, a common theme emerges: that a wide variety of anti-oxidants can ameliorate the toxic gain-of-function of A $\beta$ . This is consistent with our argument that A $\beta$  itself is the principal pro-oxidant in Alzheimer's disease.

Other approaches to alter the progression of Alzheimer's disease involve the use of oestrogen, anti-oxidants (alone or in combination with selegiline) or non-steroidal anti-inflammatory drugs (NSAIDs) (Fig. 2).

A controversial area involves the effects of hormones (oestrogens and testosterone especially) and how they may affect amyloid precursor protein metabolism. Oestrogens have been shown not only to modulate neurotransmission, but also act as free radical scavengers, activating nuclear oestrogen receptor in intracellular signalling (Behl and Holsboer 1999) and preventing A $\beta$  formation by promoting the  $\alpha$ -secretase amyloid precursor protein-nonamyloidogenic pathway (Xu *et al.* 1998). Conflicting results in experimental models have appeared, in which oestrogen deficiency exacerbates A $\beta$  in the amyloid precursor protein23 transgenic model (Yue *et al.* 2005) and neither oestrogen deprivation nor replacement affected A $\beta$  deposition in the PDAPP transgenic model (Green *et al.* 2005). The mechanisms through which oestrogen/testosterone might act remain obscure, but include oestrogen-dependent regulation of metal homeostasis in the brain through the expression of the neuronal zinc-transporter, ZnT3.

If, as postulated, Alzheimer's disease pathology is the consequence of a chronic imbalance between A $\beta$  production and clearance, the most rational strategy to treat the disease would involve retarding, halting or even reversing the process that leads to increased production of A $\beta$  (Barrow 2002).

The most promising strategy for neuroprotection might be reducing formation of A $\beta$  by partially inhibiting either  $\beta$ - or  $\gamma$ -secretase (Fig. 2) and/or stimulation of  $\alpha$ -secretase activity (Xia 2003; Zimmermann *et al.* 2004; Caccamo *et al.* 2006). Total inhibition of either  $\beta$ - or  $\gamma$ -secretase should block A $\beta$  production completely. There are already potent  $\gamma$ -secretase inhibitors (Lanz *et al.* 2004; Wong *et al.* 2004) undergoing human trials. During 2005, the first publications of *in vivo*  $\gamma$ -secretase inhibition/modulation of A $\beta$ 42 biogenesis appeared. One of the first known inhibitors (DAPT) was shown to be effective in acute experiments in behavioral tests (contextual fear conditioning) in transgenic mice (Comery *et al.* 2005). Chemical modifications to the structure of DAPT has improved its delivery to the brain (Quélever *et al.* 2005) as well as with other compounds (Laras *et al.* 2005) to achieve lower effective dosages while minimising the risk of peripheral adverse effects. Several classes of inhibitors and modulators are showing favourable acute pharmacokinetics,

with rapid lowering of plasma and CSF A $\beta$  levels (Anderson *et al.* 2005; Barten *et al.* 2005). The first in-human Phase I results have shown that LY450139 (Lilly Indianapolis, USA) achieved a significant lowering of plasma A $\beta$ , but not CSF A $\beta$ , in normal volunteers (up to 50 mg/day for 14 days) or subjects with Alzheimer's disease (up to 40 mg/day for 6 weeks) (Siemers *et al.* 2005; Siemers *et al.* 2006). The drug was well tolerated. Further explorations of the properties of  $\gamma$ -secretase inhibitors are revealing unanticipated effects on synaptic function (Dash *et al.* 2005). New classes of  $\gamma$ -secretase inhibitors/modulators continue to be disclosed, as part of the attempt to develop compounds that are devoid of side-effects (Gundersen *et al.* 2005; Lewis *et al.* 2005). The major concern is the inhibition of signalling in the Notch pathway, which affects cellular differentiation (van Es *et al.* 2005). Further research on the mechanistic operations of the  $\gamma$ -secretase complex (Sato *et al.* 2005) may lead to new paths of drug discovery, as might gene targeting of presenillin, PEN-2, APH-1 and nicastrin lead to selective regulation of  $\gamma$ -secretase activity (Saura *et al.* 2005; Xie *et al.* 2005). The development of  $\beta$ -secretase inhibitors has been focused on the discovery and design of compounds which target the active site of BACE-1. Improved assays (Pietrak *et al.* 2005) and structural-based *in silico* designs (Hanessian *et al.* 2005; Huang *et al.* 2005) have added to the existing pipe-line of drugs in early preclinical development (Kornacker *et al.* 2005; Lefranc-Jullien *et al.* 2005) or early discovery programs (Lee *et al.* 2005b). Other proteins interacting with BACE-1 may become drug targets (Xie and Guo 2005) and gene targeting of BACE-1 mRNA using siRNA is also producing encouraging preliminary results (Singer *et al.* 2005).

In contrast to the inhibition of A $\beta$  biogenesis, therapeutic strategies which directly target A $\beta$  itself should have a lower risk of unanticipated side-effects, as the accumulated A $\beta$  molecule is a disease-specific trait of Alzheimer's disease. If the A $\beta$  fragment (or its domain within amyloid precursor protein) does, however, subserve some critical normal function, then targeting A $\beta$  itself might interfere with this function and thereby lead to adverse side-effects. To date, however, a normal function for A $\beta$  has not been identified. Amyloid precursor protein knockout mice are viable and healthy, providing some support for this idea.

Given the evidence that levels of soluble A $\beta$  correlate with disease severity (McLean *et al.* 1999) and that the A $\beta$  is the main neurotoxic factor in the development of Alzheimer's disease, the design of drugs directly targeting A $\beta$  and its varied conformations as well as agents inhibiting A $\beta$  oligomerisation should be more effective than those that merely block A $\beta$  deposition (Wolfe 2002). Two basic strategies have been proposed to reduce or remove A $\beta$  from the brain: immunisation (McLaurin *et al.* 2002; Schenk 2002), breaking the pathway that leads to A $\beta$  deposition by inducing an active immune response against the A $\beta$  (Janus

*et al.* 2000; Weiner *et al.* 2000), passive administration of specific anti-A $\beta$  antibodies (Bard *et al.* 2000; DeMattos *et al.* 2001; Wilcock *et al.* 2003), promoting microglial clearance (Bard *et al.* 2000), and/or by redistribution of A $\beta$  into the systemic circulation (DeMattos *et al.* 2001) (Fig. 2). Since 1999, increasing evidence has accumulated to make a compelling antibody-mediated A $\beta$  clearance/neutralisation strategy. Experiments in amyloid precursor protein transgenic mice models continue to demonstrate efficacy without detectable toxicity (Brendza *et al.* 2005; Klyubin *et al.* 2005). The aborted clinical trial with the Elan Ab42 antigen (AN1792) has provided a wealth of clinical information (Gilman *et al.* 2005; Lee *et al.* 2005d; Masliah *et al.* 2005) which will assist further development of strategies designed to avoid the auto-immune adverse events (Lee *et al.* 2005a; Racke *et al.* 2005). Chief among these will be avoidance of T-cell-mediated responses (Agadjanyan *et al.* 2005) and the development of passive immunisation protocols (Hartman *et al.* 2005). Passive immunisation clinical trials are currently underway. In the meantime, novel methods of antigen presentation (Frenkel *et al.* 2005; Youm *et al.* 2005) and the use of neo-epitopes (Arbel *et al.* 2005; Yamamoto *et al.* 2005) are under investigation. Neo-epitopes generated post-translationally by oxidative modification of A $\beta$  should have inherently less potential to generate an auto-immune adverse reaction.

Trials of anti-inflammatories in Alzheimer's disease have been conducted, and considerable research efforts undertaken to examine the effects of anti-inflammatories in a variety of experimental models. These include the non-steroidal anti-inflammatories (Farias *et al.* 2005; Morihara *et al.* 2005), peroxisome proliferator-activated receptor- $\gamma$  agonists (Echeverria *et al.* 2005; Shie *et al.* 2005), cannabinoids (Ramirez *et al.* 2005) and glucocorticoids (Boedker *et al.* 2005). To date, no prospective clinical trial with an anti-inflammatory has shown a convincing beneficial outcome. Anti-inflammatory medication has also been shown to have direct effects on the cleavage of amyloid precursor protein by  $\gamma$ -secretase, an effect that is independent of the drugs' inhibition of cyclooxygenase and other inflammatory mediators (Behr *et al.* 2004) (Fig. 2). Some such drugs reduce cytopathology in amyloid precursor protein transgenic mice (Lim *et al.* 2001; Jantzen *et al.* 2002).

While high-cholesterol diets increase A $\beta$  pathology in experimental animals (Refolo *et al.* 2000) general caloric restriction has often been associated with longevity in rodent models of aging, and recent studies in transgenic Alzheimer's disease models (Patel *et al.* 2005; Wang *et al.* 2005) and normal rodents (Tang 2005), suggesting an effect on A $\beta$  plaque load or  $\alpha$ -secretase processing of amyloid precursor protein. This led to the assessment of the effects of modulating cholesterol homeostasis over Alzheimer's disease pathology. Cholesterol and inhibitors of cholesterol synthesis (statins) have been shown to significantly alter

amyloid precursor protein processing *in vitro*, with a reduction in  $\beta$ -secretase cleavage and a decreased A $\beta$  production (Fassbender *et al.* 2001), reducing pathology in amyloid precursor protein transgenic mice (Refolo *et al.* 2001). Statins have been associated with a lowered incidence of Alzheimer's disease (Wolozin *et al.* 2000). While some early phase clinical trials with statins have shown encouraging results (Masse *et al.* 2005), others have not (Höglund *et al.* 2005a, b). Cholesterol-independent effects have also been noted for statins acting on isoprenyl intermediates in the cholesterol biosynthetic pathways, with a putative anti-inflammatory effect induced by reactive microglia (Cole *et al.* 2005; Cordle and Landreth 2005). Statins have also been implicated in the toxic gain-of-function of A $\beta$  interacting with  $\alpha 7$ -nAChR (Si *et al.* 2005), although the mechanism for this remains unclear.

Based on the role that metal ions such as Cu, Fe and Zn play in the biochemical processes associated with A $\beta$  deposition and neurotoxicity (Cherny *et al.* 1999; Barnham *et al.* 2004b), a further therapeutic strategy based on inhibiting A $\beta$ /metal interactions led to the design and development of molecules, known as metal-protein attenuating compounds (MPACs) (Barnham *et al.* 2004b) (Fig. 2), that inhibit the deleterious effects of aberrant metal interactions through competition with the target protein for the metal ions, leading to a normalisation of metal homeostasis. MPACs not only inhibit the *in vitro* generation of hydrogen peroxide but also have been shown to reverse the precipitation of A $\beta$  *in vitro* and in postmortem human brain specimens (Bush 2002), reducing A $\beta$  amyloid burden by a direct solubilisation and by reducing toxic oxidative stress (Cherny *et al.* 2001). Clioquinol, 5-chloro-7-iodo-8-hydroxyquinoline, is a hydrophobic Zn and Cu chelator that freely crosses the blood-brain barrier (Padmanabhan *et al.* 1989). Preclinical studies showed that clioquinol increased soluble phase A $\beta$  by more than 200% in a concentration-dependent fashion in homogenised postmortem human brain samples, and its efficacy tested in transgenic Tg2576 mice showed a dramatic 49% decrease in brain A $\beta$  deposition after 9 weeks of oral treatment (Cherny *et al.* 2001; Raman *et al.* 2005). Clioquinol was chosen to be tested as an A $\beta$  amyloid solubilising and antitoxic agent in a randomised, double blind, placebo-controlled pilot Phase II clinical trial (Ritchie *et al.* 2003; Ibach *et al.* 2005). Oral clioquinol treatment was statistically significant in preventing cognitive deterioration in the moderately severe Alzheimer's disease patient group, with no evidence of toxicity (Ritchie *et al.* 2003). Other groups have considered chelators (Gaeta and Hider 2005; Liu *et al.* 2005) or other novel compounds (Cui *et al.* 2005). Our own studies have progressed with a new chemical entity based around the 8-OH quinoline structure. This compound (PBT2, Prana Biotechnology, Melbourne, Australia) has passed phase I and will soon commence phase II clinical development.

Additional binding sites on A $\beta$ , such as the glycosaminoglycan (GAG) site [HHQK (13–16)], have been targeted with compounds such as 3-amino-1-propanesulfonic acid (3-APS: Alzhemed, Neurochem Inc., Quebec, Canada). The results of early clinical trials released by the company have shown some effects on CSF A $\beta$ 42, but none on ADAS-cog or MMSE.

The pharmaceutical industry has for a long time interrogated their libraries for compounds that are anti-aggregants and/or antifibrillogenic. Many hits with compounds that look similar to Congo Red have never been developed. Similarly, compounds capable of disaggregating or defibrillating A $\beta$  have been sought, but not with the intensity of the search for anti-aggregants. Many peptidyl/protein-like designs have been examined (Gibson and Murphy 2005; Lee *et al.* 2005e; Schuster *et al.* 2005), but other small molecules have also been discovered which hold some promise (Hennessy and Buchwald 2005; Kanopathipillai *et al.* 2005; Lee *et al.* 2005c). We have identified other structural changes or mechanisms of toxicity for A $\beta$  which include the oxidative modifications of Tyr10 and Met35, the interaction of A $\beta$  with the polar head groups of the lipid bilayer, and the interaction of A $\beta$  with other proteins (Mettenburg *et al.* 2005; Yang S. P. *et al.* 2005).

There are many new potential therapeutic strategies under evaluation. With the advent of RNA interference silencing, it is to be expected that attempts at direct amyloid precursor protein gene regulation will emerge. As a forerunner to this, models in which the overexpressed human amyloid precursor protein transgene in mice can be down-regulated with doxycycline provide a proof-of-principle that rapid control over A $\beta$  expression and deposition can be obtained without gross adverse side-effects (Jankowsky *et al.* 2005). Unexpectedly, A $\beta$  deposits formed before the onset of down-regulation of APP seemed to be remarkably stable, indicating that any treatment of this type in isolation might have to be administered early in the natural history of Alzheimer's disease.

As a presumptive cell surface receptor, amyloid precursor protein probably has ligands and effector mechanisms for signal transduction. Nearly 200 proteins have been reported as having direct interactions with amyloid precursor protein. Suspected ligands in the extracellular domain include growth factors (nerve growth factor in particular), heparin-containing extracellular matrix, metals (through the extracellular Cu/Zn binding domain) and amyloid precursor protein itself through hetero- and homo-dimerisation. Small compounds such as propentofylline (Chauhan *et al.* 2005) can affect nerve growth factor release, and through this modulate the amyloidogenic pathway.

The re-uptake, clearance and degradation of A $\beta$  is still subject to considerable uncertainties. If sporadic Alzheimer's disease is the result of a low level shift (< 10%, for example) in the efficiency in any of these mechanisms, then a therapeutic strategy aimed at restoring or by-passing this

faulty mechanism could be very useful. Each of the different pools of A $\beta$  probably has slightly different mechanisms of elimination, varying with the cellular compartment in which A $\beta$  resides over the course of its catabolic cycle. Several pieces of evidence point towards the enzymes neprilysin and insulin degrading enzyme as key players (Saito *et al.* 2005) but the highly sought evidence from gene linkage studies remains elusive (Eckman and Eckman 2005). A new candidate, angiotensin-converting enzyme (ACE), has emerged (Hemming and Selkoe 2005) and it will be of great interest to learn whether the ACE-inhibitors could be having an adverse influence over the natural history of Alzheimer's disease.

While A $\beta$  has captured the imagination of most Alzheimer's disease researchers, studies of the neurofibrillary tangle and its constituent, the tau microtubule associated protein, have progressed to a point where clear therapeutic strategies are emerging. The exact form of tau which causes neuronal degeneration is now being re-examined (Duff and Planel 2005), with data emerging that the soluble aggregated species, akin to soluble A $\beta$  oligomers, might represent the best target. The binding sites on tau (Mukrasch *et al.* 2005) for a variety of interactors are potential targets. Down-regulation of expression of the tau gene (Santacruz *et al.* 2005) or changing the alternative splicing (Rodriguez-Martin *et al.* 2005) also offer some new strategies. As the molecular basis for the accumulation of tau in the Alzheimer's disease brain becomes clearer, so will more precise therapeutic targets. If tau accumulation is closely linked to A $\beta$  toxicity, then oxidative modifications of tau become understandable (Santa-María *et al.* 2005) and subject to anti-oxidative classes of drugs. Metal ions might also affect this pathway (Ma *et al.* 2006). Looking at the normal function and processing of tau has raised the possibility of using microtubule-stabilising agents such as paclitaxel (Taxol) (Michaelis *et al.* 2005). Great controversy still persists about the role of normal and abnormal phosphorylation of tau in its passage from a highly soluble cytoskeletal-associated protein into an aggregated neurofibrillary tangle. If phosphorylation of specific amino acids by specific kinases such as c-Abl (Derkinderen *et al.* 2005), Cdk5 (Sakaue *et al.* 2005), GSK-3 (Noble *et al.* 2005) or MAPK (Lambourne *et al.* 2005) proves to be pathogenic, then specific kinase inhibitors, including lithium (Noble *et al.* 2005), might be developed for Alzheimer's disease. However, if phosphorylation proves to be a secondary event, following aggregation and accumulation of intracellular tau, then this approach would not be expected to be useful. Other post-translational modifications including proteolytic cleavages have been proposed (Cotman *et al.* 2005) – all amenable to therapeutic drug discoveries. As with A $\beta$ , small compounds capable of inhibiting aggregation and fibrillisation of tau are now being examined *in vitro* (Necula *et al.* 2005; Taniguchi *et al.* 2005) but require much more work in animal models.

It is extremely unlikely that a single class of compound or targeting a single mechanism of action will be sufficient to treat Alzheimer's disease. For this complex disease, it is far more likely that a combination of drugs targeting various aspects of the greater amyloid precursor protein/A $\beta$  pathway will evolve into some form of rational therapy.

## Neuroimaging

The insights into the molecular mechanism of Alzheimer's disease pathogenesis have opened new opportunities not only for the successful development of neuroprotective treatment strategies aimed at the prevention of A $\beta$  generation but also into new structural and functional neuroimaging approaches.

Structural neuroimaging techniques, such as computed tomography and magnetic resonance imaging, are routinely used in the clinical evaluation of Alzheimer's disease patients, and are mainly used to exclude other treatable causes of dementia (Scheltens 2001). Widespread cortical atrophy with a thinning of medial temporal lobe structures are the most consistent structural neuroimaging findings associated with Alzheimer's disease, though not pathognomonic of the disease because there is overlap with 'normal' aging (Jobst *et al.* 1992). To account for the cognitive impairment commonly observed in Alzheimer's disease, magnetic resonance imaging has been used to examine atrophy of the entorhinal, perirhinal and temporal cortices in patients with early Alzheimer's disease (Chetelat and Baron 2003; Thompson *et al.* 2004). Volumetric changes on magnetic resonance imaging are entirely consistent with the patterns of neuropathological progression in Alzheimer's disease, and the severity of volume loss is correlated with disease severity. The same regions were found to be reduced in both mild cognitive impairment and Alzheimer's disease compared to controls (Du *et al.* 2001; de Leon *et al.* 2006). Both conditions were also significantly associated with cortical grey matter loss and ventricular enlargement. Substantial neuronal loss has occurred by the time atrophy is detectable by MRI (Killiany *et al.* 2002). Furthermore, the absence of cortical atrophy or medial temporal lobe changes is not sufficient to exclude a diagnosis of Alzheimer's disease. The fact that structural changes at visual inspection are not evident until late in the course of the disease has prompted the development and refinement of more sophisticated quantitative techniques, capable of revealing subtle changes over time (Petrella *et al.* 2003).

Modern functional neuroimaging techniques such as positron emission tomography (PET), single photon emission tomography (SPECT), magnetic resonance spectroscopy (MRS), functional MRI, MR Diffusion weighted imaging and magnetoencephalography (MEG) have been developing new approaches not only to determine if an individual suffers from a particular form of dementia, but also delving into the molecular mechanisms of synaptic failure and neurodegen-

eration (Schuff *et al.* 2002; Petrella *et al.* 2003). More sensitive than structural imaging modalities, functional neuroimaging approaches have the capability to identify subtle pathophysiological changes in the brain before structural changes are present (Xu *et al.* 2000; Dickerson *et al.* 2001), therefore possessing greater potential for accurate and early diagnosis, monitoring disease progression, and better treatment follow-up (Silverman and Phelps 2001; Villemagne *et al.* 2005a).

Perfusion assessed by functional MRI has been shown to have about 85–95% sensitivity for the diagnosis of patients with mild or moderate Alzheimer's disease with 88–95% specificity (Bozzao *et al.* 2001). Proton MRS studies (Doraiswamy *et al.* 2000) have shown regional decreases in N-acetylaspartate in patients with Alzheimer's disease in temporal and parietal cortices (Jessen *et al.* 2000) demonstrating a positive correlation between the degree of N-acetylaspartate reductions and disease severity by neuropathologic criteria (Mohanakrishnan *et al.* 1995). MRS has also been applied to monitor response to therapeutic interventions in Alzheimer's disease (Satlin *et al.* 1997). Patients with Alzheimer's disease showed significantly higher mean diffusibility in hippocampus, cingulate, temporal and parietal white matter than a control group using diffusion weighted MR imaging (Kantarci *et al.* 2001), while diffusion tensor MR imaging has shown diffuse reduction in white matter integrity in patients with Alzheimer's disease (Rose *et al.* 2000).

PET is a sensitive molecular imaging technique that allows *in vivo* quantification of radiotracer concentrations in the picomolar range, permitting detection of disease processes at asymptomatic stages when there is no evidence of anatomic changes on CT and MRI (Phelps 2000).

Several studies have evaluated regional cerebral glucose metabolism with fluorodeoxyglucose (FDG) and PET. A typical pattern of reduced temporoparietal FDG uptake with sparing of the basal ganglia, thalamus, cerebellum and primary sensorimotor cortex is typical of Alzheimer's disease (Coleman 2005). Though FDG-PET is mainly used in the differential diagnosis of Alzheimer's disease, it is the neuroimaging technique that has been shown to yield the highest prognostic value for providing a diagnosis of pre-symptomatic Alzheimer's disease 2 years or more before the full dementia picture is manifested (Silverman *et al.* 2001; Chang and Silverman 2004). In a multicentre study the prognostic value of FDG-PET showed a high degree of sensitivity (93%) and moderate specificity (73%) for prediction of progressive dementia (Silverman *et al.* 2001).

SPECT studies evaluating regional cerebral blood flow (rCBF) have shown a similar pattern as the one described for PET-FDG studies, with relative rCBF paucity in the temporoparietal regions (Camargo 2001). In a prospective study with histologic confirmation of over 200 dementia cases and 119 control cases (Jobst *et al.* 1998), SPECT rCBF evalu-

ation allowed differentiation of patients with Alzheimer's disease from control subjects with high sensitivity and specificity (89% and 80%, respectively).

PET and SPECT can also assess neurotransmitter/neuro-receptor systems *in vivo*. Abnormally low densities of nAChRs have been measured *in vitro* in autopsy brain tissue of Alzheimer's disease patients. PET studies revealed a reduced uptake and binding of  $^{11}\text{C}$ -nicotine in the temporal and frontal cortices of Alzheimer's disease patients (Nordberg 1993). Tacrine treatment increased cerebral blood flow, cerebral glucose utilisation, and uptake of  $^{11}\text{C}$ -nicotine to the brain paralleled by improvement in neuropsychological performance (Nordberg *et al.* 1998). Though the main focus of neuroreceptor studies in Alzheimer's disease has been the study of nAChRs, several other neurotransmitter/neuro-receptor systems were also evaluated in dementing neurodegenerative diseases (Higuchi *et al.* 2000; Walker *et al.* 2002; Piggott *et al.* 2003; Kepe *et al.* 2006).

As mentioned before, because new treatment strategies to prevent or slow disease progression through early intervention are being developed and implemented, there is an urgent need for early disease recognition, which is reflected in the necessity of developing sensitive and specific biomarkers, specific for a particular trait underlying the pathological process, as adjuncts to clinical and neuropsychological tests. Clinical criteria together with current structural neuroimaging techniques (CT or MRI) are sensitive and specific enough for the diagnosis of Alzheimer's disease at the mid or late stages of the disease; however, they focus on non-specific features derived mainly from neuronal loss and atrophy, which are late features in the progression of the disease, and are secondary to the basic functional alteration. The development of a reliable method of assessing disease-specific biomarkers, such as A $\beta$  amyloid burden *in vivo*, may permit early diagnosis at presymptomatic stages and more accurate differential diagnosis, while also allowing treatment follow-up.

In the same way neuropathology was boosted by the techniques and dyes introduced by visionary pioneers like Cajal and Nissl, we are now seeing some derivatives of those histological dyes finding their way into emission tomography (Sair *et al.* 2004; Villemagne *et al.* 2005a) and magnetic resonance imaging (Sato *et al.* 2004; Zhang *et al.* 2004; Higuchi *et al.* 2005).

As A $\beta$  is at the centre of pathogenesis of Alzheimer's disease, most efforts were focussed on developing radiotracers or agents that allow A $\beta$  imaging *in vivo* (Sair *et al.* 2004; Villemagne *et al.* 2005a). Tau imaging is still in its early stages of development (Okamura *et al.* 2005).

Several compounds have been evaluated as potential A $\beta$  probes: derivatives of histopathological dyes such as Congo red, Chrysamine-G, Thioflavin S and T, and acridine orange (Mathis *et al.* 2002; Klunk *et al.* 2003; Shimadzu *et al.* 2003; Zhang *et al.* 2005), NSAID derivatives (Shoghi-Jadid

*et al.* 2002) as well as self-associating A $\beta$  amyloid fragments (Marshall *et al.* 2002), anti-A $\beta$  monoclonal antibodies (Walker *et al.* 1994), serum amyloid p, and basic fibroblast growth factor (Shi *et al.* 2002). Some of these PET radiotracers are being evaluated in transgenic mice as potential MR contrast agents (Sato *et al.* 2004; Higuchi *et al.* 2005; Vanhoutte *et al.* 2005; Wadghiri *et al.* 2005).

Quantitative imaging of A $\beta$  burden *in vivo* is allowing us to define the relationship between A $\beta$  burden and clinical and neuropsychological characteristics in Alzheimer's disease. <sup>11</sup>C-PIB, a derivative of Thioflavin T, has been shown to possess high affinity and high specificity for amyloid fibrils and binds to amyloid plaque but not neurofibrillary tangles in postmortem human brain homogenates *in vitro* (Lockhart *et al.* 2005; Ye *et al.* 2005). PET studies in human subjects have shown a robust difference between the retention pattern in Alzheimer's disease patients and healthy controls, with Alzheimer's disease cases showing significantly higher retention of <sup>11</sup>C-PIB in neocortical areas of the brain affected by A $\beta$  deposition (Klunk *et al.* 2004; Price *et al.* 2005; Villemagne *et al.* 2005b). A $\beta$  burden is significantly elevated in Alzheimer's disease, dementia with Lewy-bodies, and about 50% of mild cognitive impairment subjects compared to healthy controls, while frontotemporal dementia and non-demented Parkinson's disease subjects show no cortical <sup>11</sup>C-PIB binding (Fig. 3). About 25% of the healthy controls showed cortical binding, predominantly in the prefrontal cortex, though to a lesser degree than Alzheimer's disease patients. The demonstration of <sup>11</sup>C-PIB binding in a proportion of healthy control subjects supports *in vitro* observations that A $\beta$  aggregation predominantly occurs before onset of dementia (Price and Morris 1999; Morris and Price 2001).

PIB PET shows promise in the differential diagnosis of Alzheimer's disease from frontotemporal dementia (Fig. 3) but the emphasis on amyloid imaging should not be limited to its capability for differential diagnosis. With new

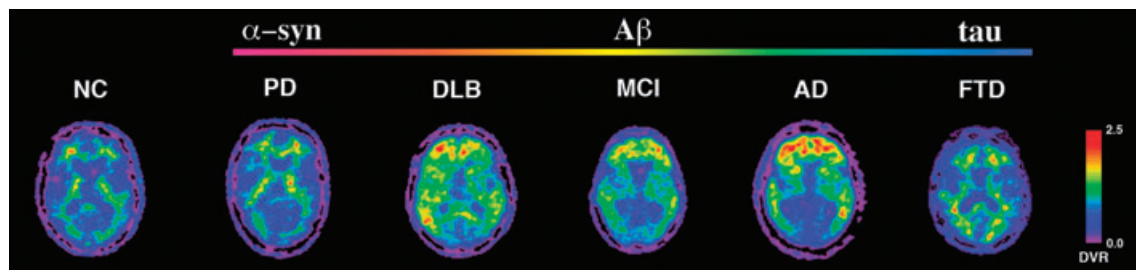
treatments to prevent or slow Alzheimer's disease progression by either preventing A $\beta$  deposition or increasing its clearance entering clinical trials, agents that could delay the onset of dementia, and the role of imaging and quantifying A $\beta$  burden *in vivo* are becoming crucial (Ritchie *et al.* 2003; Schenk *et al.* 2004). The ability to detect preclinical or early stage disease through clinical, laboratory and neuroimaging tests, combined with anti-A $\beta$  amyloid in the at-risk patient, or the patient with mild cognitive impairment, may prevent or delay functional and irreversible cognitive losses, making it possible at the same time to customise and monitor treatment.

## Conclusions

Alzheimer's disease is a neurodegenerative disorder characterised by a slow but relentless progressive cognitive decline and memory loss. It has a devastating effect not only on the sufferer but also on their caregivers, with a tremendous socio-economic impact not only on families but also on the health system, which will only increase in the upcoming years.

The neuropathologic hallmarks of the disease are extracellular deposits of A $\beta$  in senile plaques, neurofibrillary tangles, with selective neuronal and synaptic loss in cortical areas of the brain associated with cognitive and memory functions.

A $\beta$  is the main component of the amyloid plaques. All available evidence points to the breakdown of A $\beta$  homeostasis as the key role in Alzheimer's disease pathogenesis. Genetic studies have shed light on the pathogenesis and progression of Alzheimer's disease. To date, four genes have been linked to autosomal dominant, early onset familial Alzheimer's disease: amyloid precursor protein, presenilin 1, presenilin 2 and apolipoprotein E. All mutations linked to amyloid precursor protein and presenilin proteins lead to an increase in A $\beta$  production. A $\beta$  not only aggregates into



**Fig. 3** Parametric PIB PET distribution volume ratio images (DVR) of a spectrum of neurodegenerative diseases secondary to misfolded proteins ( $\alpha$ -syn, A $\beta$ , tau). Representative PET images of a 73-year-old healthy control (NC) subject (MMSE 30), a 61-year-old Parkinson's disease (PD) patient (MMSE 27), a 78-year-old dementia with Lewy-body dementia (DLB) patient (MMSE 19), a 70-year-old mild cognitive

impairment (MCI) patient (MMSE 26), an 82-year-old Alzheimer's disease (AD) patient (MMSE 22) and a 78-year-old frontotemporal dementia (FTD) patient (MMSE 26). DVR PET images show no cortical PIB retention in NC, PD or FTD with a clearly different pattern from DLB, MCI or AD patients, and significant PIB retention in the frontal and temporal cortices.

amyloid plaques but is toxic *per se*, while having an effect on intracellular tangle formation and other factors (e.g. cytokines, neurotoxins, etc.) that also play an important role in the neurotoxic progression of Alzheimer's disease.

A $\beta$  is neurotoxic through a number of possible mechanisms including oxidative stress, excitotoxicity, energy depletion, inflammatory response and apoptosis, and whereas the exact mechanism by which A $\beta$  might produce synaptic loss and neuronal death is controversial, it is believed that a toxic oxidative interaction between various metal species and A $\beta$  triggers an oxidative response with free radical production, progressive disruption of synaptic and neuronal function leading ultimately to cell death.

At this point there is no cure for Alzheimer's disease. A deeper understanding of the molecular mechanism of A $\beta$  formation, degradation and neurotoxicity is being translated into new neuroimaging and therapeutic approaches. Most of the approved palliative treatments regimens involve the use of acetylcholinesterase inhibitors, glutamatergic agents, non-steroidal anti-inflammatory drugs and anti-oxidants. The clinical development of drugs directly targeting the A $\beta$  pathway is at an early stage. The most promising approaches focus on reducing A $\beta$  formation, increasing its removal or blocking the formation of A $\beta$  oligomers and fibrils, therefore inhibiting neurotoxicity. The  $\gamma$ -secretase inhibitors trials are of immense theoretical interest, as they are likely to provide the most compelling support for the A $\beta$  theory of Alzheimer's disease. The trials around the A $\beta$  metal binding site or the CAG binding sites also have the potential to address this aspect. Immunisation/immunomodulation of A $\beta$  holds great promise for elucidating the A $\beta$  clearance/neutralisation strategies on which there is currently a dearth of information. A variety of prospective statin-mediated approaches will also test the hypothesis that cholesterol has an important role in the biogenesis of Alzheimer's disease. The anti-oxidant trials have the disadvantage of lacking specificity for A $\beta$ , but nonetheless will continue to provide much needed guidance for the general theory of the Alzheimer's disease brain being under oxidative stress.

Currently, clinical diagnosis of Alzheimer's disease is based on progressive impairment of memory and decline in at least one other cognitive domain, and by excluding other diseases using structural neuroimaging techniques (CT or MRI). This approach is only sensitive and specific enough for the diagnosis of Alzheimer's disease at the mid or late stages of the disease. Because new treatment strategies to prevent or slow disease progression through early intervention are being developed and implemented, there is an urgent need for early disease recognition, which is reflected in the necessity of developing sensitive and specific biomarkers, specific for a particular trait underlying the pathological process, as adjuncts to clinical and neuropsychological tests.

The development of a reliable method of assessing A $\beta$  amyloid burden *in vivo* may permit early diagnosis at

presymptomatic stages and more accurate differential diagnosis, while also allowing treatment follow-up. *In vivo* amyloid imaging with PET is allowing new insights into A $\beta$  deposition in the brain, facilitating research into the causes, diagnosis and future treatment of dementias, where A $\beta$  may play a role.

Following Alois Alzheimer's groundbreaking presentation in Tübingen 100 years ago (Alzheimer 1907) it has been a long night's journey into the day. We are now entering a new dawn that promises the delivery of revolutionary developments for the control of dementias.

### Acknowledgements

We thank Christopher Rowe, Catriona McLean, Steven Ng, Michelle Fodero-Tavoletti, Tiffany Cowie, Lisa Foster, Laura Leone, Fairlie Hinton and Emma Mitchell for their crucial role in our ongoing research projects.

We apologise to our colleagues for omitting references due to space constraints.

We acknowledge the funding support of the National Health and Medical Research Council of Australia, Neurosciences Victoria, Austin Hospital Medical Research Foundation, Schering AG and Prana Biotechnology Ltd.

### References

- Agadjanyan M. G., Ghochikyan A., Petrushina I., Vasilevko V., Movsesyan N., Mkrtichyan M., Saing T. and Cribbs D. H. (2005) Prototype Alzheimer's disease vaccine using the immunodominant B cell epitope from beta-amyloid and promiscuous T cell epitope pan HLA DR-binding peptide. *J. Immunol.* **174**, 1580–1586.
- Aguado-Llera D., Arilla-Ferreiro E., Campos-Barros A., Puebla-Jimenez L. and Barrios V. (2005) Protective effects of insulin-like growth factor-I on the somatostatinergic system in the temporal cortex of beta-amyloid-treated rats. *J. Neurochem.* **92**, 607–615.
- Allen R. G. and Tresini M. (2000) Oxidative stress and gene regulation. *Free Radic. Biol. Med.* **28**, 463–499.
- Alzheimer A. (1907) Über eine eigenartige Erkrankung der Hirnrinde. *Allg. Z. Psychiatr.* **64**, 146–148.
- Andersen O. M., Reiche J., Schmidt V., *et al.* (2005) Neuronal sorting protein-related receptor sorLA/LR11 regulates processing of the amyloid precursor protein. *Proc. Natl Acad. Sci. U S A* **102**, 13 461–13 466.
- Andersen O. M., Schmidt V., Spoelgen R., *et al.* (2006) Molecular dissection of the interaction between amyloid precursor protein and its neuronal trafficking receptor SorLA/LR11. *Biochemistry* **45**, 2618–2628.
- Anderson J. J., Holtz G., Baskin P. P., *et al.* (2005) Reductions in beta-amyloid concentrations *in vivo* by the gamma-secretase inhibitors BMS-289948 and BMS-299897. *Biochem. Pharmacol.* **69**, 689–698.
- Arbel M., Yacoby I. and Solomon B. (2005) Inhibition of amyloid precursor protein processing by {beta}-secretase through site-directed antibodies. *Proc. Natl Acad. Sci. U S A* **102**, 7718–7723.
- Arispe N., Rojas E. and Pollard H. B. (1993) Alzheimer disease amyloid beta protein forms calcium channels in bilayer membranes: blockade by tromethamine and aluminum. *Proc. Natl Acad. Sci. U S A* **90**, 567–571.

- Arlt S., Beisiegel U. and Kontush A. (2002) Lipid peroxidation in neurodegeneration: new insights into Alzheimer's disease. *Curr. Opin. Lipidol.* **13**, 289–294.
- Atwood C. S., Moir R. D., Huang X., Scarpa R. C., Bacarra N. M., Romano D. M., Hartshorn M. A., Tanzi R. E. and Bush A. I. (1998) Dramatic aggregation of Alzheimer A $\beta$  by Cu (II) is induced by conditions representing physiological acidosis. *J. Biol. Chem.* **273**, 12 817–12 826.
- Auld D. S., Komecook T. J., Bastianetto S. and Quirion R. (2002) Alzheimer's disease and the basal forebrain cholinergic system: relations to beta-amyloid peptides, cognition, and treatment strategies. *Prog. Neurobiol.* **68**, 209–245.
- Bales K. R., Verina T., Dodel R. C., *et al.* (1997) Lack of apolipoprotein E dramatically reduces amyloid beta-peptide deposition. *Nat Genet* **17**, 263–264.
- Bano S. and Parihar M. S. (1997) Reduction of lipid peroxidation in different brain regions by a combination of alpha-tocopherol and ascorbic acid. *J. Neural Transm* **104**, 1277–1286.
- Bard F., Cannon C., Barbour R., *et al.* (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.
- Barnham K. J., McKinsty W. J., Multhaup G., *et al.* (2003a) Structure of the Alzheimer's disease amyloid precursor protein copper binding domain. A regulator of neuronal copper homeostasis. *J. Biol. Chem.* **278**, 17 401–17 407.
- Barnham K. J., Ciccotosto G. D., Tickler A. K., *et al.* (2003b) Neurotoxic, redox-competent Alzheimer's beta-amyloid is released from lipid membrane by methionine oxidation. *J. Biol. Chem.* **278**, 42 959–42 965.
- Barnham K. J., Haeflner F., Ciccotosto G. D., *et al.* (2004a) Tyrosine gated electron transfer is key to the toxic mechanism of Alzheimer's disease beta-amyloid. *FASEB J.* **18**, 1427–1429.
- Barnham K. J., Masters C. L. and Bush A. I. (2004b) Neurodegenerative diseases and oxidative stress. *Nat. Rev. Drug Discov.* **3**, 205–214.
- Barrow C. J. (2002) Advances in the development of Abeta-related therapeutic strategies for Alzheimer's disease. *Drug News Perspect.* **15**, 102–109.
- Barten D. M., Guss V. L., Corsa J. A., *et al.* (2005) Dynamics of {beta}-amyloid reductions in brain, cerebrospinal fluid, and plasma of {beta}-amyloid precursor protein transgenic mice treated with a {gamma}-secretase inhibitor. *J. Pharmacol. Exp. Ther.* **312**, 635–643.
- Bayer T. A., Schafer S., Simons A., *et al.* (2003) Dietary Cu stabilizes brain superoxide dismutase 1 activity and reduces amyloid Abeta production in APP23 transgenic mice. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 14 187–14 192.
- Beffert U. and Poirier J. (1998) ApoE associated with lipid has a reduced capacity to inhibit beta-amyloid fibril formation. *Neuroreport* **9**, 3321–3323.
- Behr D., Clarke E. E., Wrigley J. D., Martin A. C., Nadin A., Churcher I. and Shearman M. S. (2004) Selected non-steroidal anti-inflammatory drugs and their derivatives target gamma-secretase at a novel site-evidence for an allosteric mechanism. *J. Biol. Chem.* **279**, 43 419–43 426.
- Behl C. and Holsboer F. (1999) The female sex hormone oestrogen as a neuroprotectant. *Trends Pharmacol. Sci.* **20**, 441–444.
- Belluti F., Rampa A., Piazzini L., *et al.* (2005) Cholinesterase inhibitors: xanthostigmine derivatives blocking the acetylcholinesterase-induced beta-amyloid aggregation. *J. Med. Chem.* **48**, 4444–4456.
- Bentahir M., Nyabi O., Verhamme J., Tolia A., Horre K., Wiltfang J., Esselmann H. and De Strooper B. (2006) Presenilin clinical mutations can affect gamma-secretase activity by different mechanisms. *J. Neurochem.* **96**, 732–742.
- Bernheimer A. W., Robinson W. G., Linder R., Mullins D., Yip Y. K., Cooper N. S., Seidman I. and Uwajima T. (1987) Toxicity of enzymically-oxidized low-density lipoprotein. *Biochem. Biophys. Res. Commun.* **148**, 260–266.
- Bertram L., Blacker D., Mullin K., *et al.* (2000) Evidence for genetic linkage of Alzheimer's disease to chromosome 10q. *Science* **290**, 2302–2303.
- Blanchard B. J., Chen A., Rozeboom L. M., Stafford K. A., Weigle P. and Ingram V. M. (2004) Efficient reversal of Alzheimer's disease fibril formation and elimination of neurotoxicity by a small molecule. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 14 326–14 332.
- Boedker M., Boetkjaer A., Bazan N. G., Cui J. G., Zhao Y., Pelaez R. P. and Lukiw W. J. (2005) Budesonide epimer R., LAU-8080 and phenyl butyl nitrone synergistically repress cyclooxygenase-2 induction in [IL-1beta+Abeta42]-stressed human neural cells. *Neurosci. Lett.* **380**, 176–180.
- Borchardt T., Camakaris J., Cappai R., Masters C. L., Beyreuther K. and Multhaup G. (1999) Copper inhibits beta-amyloid production and stimulates the non-amyloidogenic pathway of amyloid-precursor-protein secretion. *Biochem. J.* **344**, Part 2, 461–467.
- Bozzao A., Floris R., Baviera M. E., Apruzzese A. and Simonetti G. (2001) Diffusion and perfusion MR imaging in cases of Alzheimer's disease: correlations with cortical atrophy and lesion load. *AJNR Am. J. Neuroradiol.* **22**, 1030–1036.
- Braak H. and Braak E. (1991) Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol (Berl)* **82**, 239–259.
- Brendza R. P., Bacsikai B. J., Cirrito J. R., *et al.* (2005) Anti-A $\beta$  antibody treatment promotes the rapid recovery of amyloid-associated neuritic dystrophy in PDAPP transgenic mice. *J. Clin. Invest.* **115**, 428–433.
- Brown G. C. and Bal-Price A. (2003) Inflammatory neurodegeneration mediated by nitric oxide, glutamate, and mitochondria. *Mol Neurobiol.* **27**, 325–355.
- Bush A. I. (2002) Metal complexing agents as therapies for Alzheimer's disease. *Neurobiol. Aging* **23**, 1031–1038.
- Bush A. I. (2003) The metallobiology of Alzheimer's disease. *Trends Neurosci.* **26**, 207–214.
- Bush A. I., Masters C. L. and Tanzi R. E. (2003) Copper, beta-amyloid, and Alzheimer's disease: tapping a sensitive connection. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 1193–1194.
- Butterfield D. A. and Boyd-Kimball D. (2004) Amyloid beta-peptide (1–42) contributes to the oxidative stress and neurodegeneration found in Alzheimer disease brain. *Brain Pathol.* **14**, 426–432.
- Butterfield D. A., Drake J., Pocerich C. and Castegna A. (2001) Evidence of oxidative damage in Alzheimer's disease brain: central role for amyloid beta-peptide. *Trends Mol. Med.* **7**, 548–554.
- Byrne E. (2002) Does mitochondrial respiratory chain dysfunction have a role in common neurodegenerative disorders? *J. Clin. Neurosci.* **9**, 497–501.
- Caccamo A., Oddo S., Billings L. M., Green K. N., Martinez-Coria H., Fisher A. and LaFerla F. M. (2006) M1 receptors play a central role in modulating AD-like pathology in transgenic mice. *Neuron* **49**, 671–682.
- Cadenas E. and Davies K. J. (2000) Mitochondrial free radical generation, oxidative stress, and aging. *Free Radic. Biol. Med.* **29**, 222–230.
- Camargo E. E. (2001) Brain SPECT in neurology and psychiatry. *J. Nucl. Med.* **42**, 611–623.
- Cappai R. and White A. R. (1999) Amyloid beta. *Int. J. Biochem. Cell Biol.* **31**, 885–889.
- Caraci F., Chisari M., Frasca G., *et al.* (2005) Nicergoline, a drug used for age-dependent cognitive impairment, protects cultured neurons against beta-amyloid toxicity. *Brain Res.* **1047**, 30–37.

- Chang C. Y. and Silverman D. H. (2004) Accuracy of early diagnosis and its impact on the management and course of Alzheimer's disease. *Expert Rev. Mol. Diagn.* **4**, 63–69.
- Chang J. Y., Chavis J. A., Liu L. Z. and Drew P. D. (1998) Cholesterol oxides induce programmed cell death in microglial cells. *Biochem. Biophys. Res. Commun.* **249**, 817–821.
- Chauhan N. B., Siegel G. J. and Feinstein D. L. (2005) Propentofylline attenuates tau hyperphosphorylation in Alzheimer's Swedish mutant model Tg2576. *Neuropharmacology* **48**, 93–104.
- Cherny R. A., Legg J. T., McLean C. A., *et al.* (1999) Aqueous dissolution of Alzheimer's disease A $\beta$  amyloid deposits by biometal depletion. *J. Biol. Chem.* **274**, 23 223–23 228.
- Cherny R. A., Atwood C. S., Xilinas M. E., *et al.* (2001) Treatment with a copper-zinc chelator markedly and rapidly inhibits  $\beta$ -amyloid accumulation in Alzheimer's disease transgenic mice. *Neuron* **30**, 665–676.
- Chetelat G. and Baron J. C. (2003) Early diagnosis of Alzheimer's disease; contribution of structural neuroimaging. *Neuroimage* **18**, 525–541.
- Citron M., Oltsersdorf T., Haass C., McConlogue L., Hung A. Y., Seubert P., Vigo-Pelfrey C., Lieberburg I. and Selkoe D. J. (1992) Mutation of the beta-amyloid precursor protein in familial Alzheimer's disease increases beta-protein production. *Nature* **360**, 672–674.
- Cole S. L., Grudzien A., Manhart I. O., Kelly B. L., Oakley H. and Vassar R. (2005) Statins cause intracellular accumulation of amyloid precursor protein, beta-secretase-cleaved fragments, and amyloid beta-peptide via an isoprenoid-dependent mechanism. *J. Biol. Chem.* **280**, 18 755–18 770.
- Coleman R. E. (2005) Positron emission tomography diagnosis of Alzheimer's disease. *Neuroimaging Clin. N. Am.* **15**, 837–846.
- Comery T. A., Martone R. L., Aschmies S., *et al.* (2005) Acute gamma-secretase inhibition improves contextual fear conditioning in the Tg2576 mouse model of Alzheimer's disease. *J. Neurosci.* **25**, 8898–8902.
- Corder E. H., Saunders A. M., Strittmatter W. J., Schmechel D. E., Gaskell P. C., Small G. W., Roses A. D., Haines J. L. and Pericak-Vance M. A. (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* **261**, 921–923.
- Cordle A. and Landreth G. (2005) 3-Hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors attenuate beta-amyloid-induced microglial inflammatory responses. *J. Neurosci.* **25**, 299–307.
- Cotman C. W., Poon W. W., Rissman R. A. and Blurton-Jones M. (2005) The role of caspase cleavage of tau in Alzheimer disease neuropathology. *J. Neuropathol. Exp. Neurol.* **64**, 104–112.
- Cui Z., Lockman P. R., Atwood C. S., Hsu C. H., Gupte A., Allen D. D. and Mumper R. J. (2005) Novel D-penicillamine carrying nanoparticles for metal chelation therapy in Alzheimer's and other CNS diseases. *Eur. J. Pharm. Biopharm.* **59**, 263–272.
- Curtain C. C., Ali F., Volitakis I., *et al.* (2001) Alzheimer's disease amyloid- $\beta$  binds copper and zinc to generate an allosterically ordered membrane-penetrating structure containing superoxide dismutase-like subunits. *J. Biol. Chem.* **276**, 20 466–20 473.
- Dash P. K., Moore A. N. and Orsi S. A. (2005) Blockade of gamma-secretase activity within the hippocampus enhances long-term memory. *Biochem. Biophys. Res. Commun.* **338**, 777–782.
- 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. U.S.A.* **98**, 8850–8855.
- Derkinderen P., Scales T. M., Hanger D. P., *et al.* (2005) Tyrosine 394 is phosphorylated in Alzheimer's paired helical filament tau and in fetal tau with c-Abl as the candidate tyrosine kinase. *J. Neurosci.* **25**, 6584–6593.
- De Strooper B., Saftig P., Craessaerts K., Vanderstichele H., Guhde G., Annaert W., Von Figura K. and Van Leuven F. (1998) Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature* **391**, 387–390.
- Dickerson B. C., Goncharova I., Sullivan M. P., Forchetti C., Wilson R. S., Bennett D. A., Beckett L. A. and deToledo-Morrell L. (2001) MRI-derived entorhinal and hippocampal atrophy in incipient and very mild Alzheimer's disease. *Neurobiol. Aging* **22**, 747–754.
- Dizdaroglu M. (1992) Oxidative damage to DNA in mammalian chromatin. *Mutat. Res.* **275**, 331–342.
- Dong J., Atwood C. S., Anderson V. E., Siedlak S. L., Smith M. A., Perry G. and Carey P. R. (2003) Metal binding and oxidation of amyloid-beta within isolated senile plaque cores: Raman microscopic evidence. *Biochemistry* **42**, 2768–2773.
- Doraiswamy P. M., Chen J. G. and Charles H. C. (2000) Brain magnetic resonance spectroscopy: role in assessing outcomes in Alzheimer's disease. *CNS Drugs* **14**, 457–472.
- Du, A. T., Schuff N., Amend D., *et al.* (2001) Magnetic resonance imaging of the entorhinal cortex and hippocampus in mild cognitive impairment and Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry* **71**, 441–447.
- Duff K. and Planel E. (2005) Untangling memory deficits. *Nat. Med.* **11**, 826–827.
- Dulubova I., Ho A., Huryeva I., Sudhof T. C. and Rizo J. (2004) Three-dimensional structure of an independently folded extracellular domain of human amyloid-beta precursor protein. *Biochemistry* **43**, 9583–9588.
- Echeverria V., Clerman A. and Doré S. (2005) Stimulation of PGE receptors EP2 and EP4 protects cultured neurons against oxidative stress and cell death following beta-amyloid exposure. *Eur. J. Neurosci.* **22**, 2199–2206.
- Eckert G. P., Wood W. G. and Muller W. E. (2005) Membrane disordering effects of beta-amyloid peptides. *Subcell. Biochem.* **38**, 319–337.
- Eckman E. A. and Eckman C. B. (2005) Abeta-degrading enzymes: modulators of Alzheimer's disease pathogenesis and targets for therapeutic intervention. *Biochem. Soc. Trans.* **33**, 1101–1105.
- Emilien G., Beyreuther K., Masters C. L. and Maloteaux J. M. (2000) Prospects for pharmacological intervention in Alzheimer disease. *Arch. Neurol.* **57**, 454–459.
- van Es J. H., van Gijn M. E., Riccio O., *et al.* (2005) Notch/gamma-secretase inhibition turns proliferative cells in intestinal crypts and adenomas into goblet cells. *Nature* **435**, 959–963.
- Farias G. G., Godoy J. A., Vazquez M. C., Adani R., Meshulam H., Avila J., Amitai G. and Inestrosa N. C. (2005) The anti-inflammatory and cholinesterase inhibitor bifunctional compound IBU-PO protects from beta-amyloid neurotoxicity by acting on Wnt signaling components. *Neurobiol. Dis.* **18**, 176–183.
- Fassbender K., Simons M., Bergmann C., *et al.* (2001) Simvastatin strongly reduces levels of Alzheimer's disease beta-amyloid peptides Abeta 42 and Abeta 40 in vitro and in vivo. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 5856–5861.
- Frenkel D., Maron R., Burt D. S. and Weiner H. L. (2005) Nasal vaccination with a proteosome-based adjuvant and glatiramer acetate clears beta-amyloid in a mouse model of Alzheimer disease. *J. Clin. Invest.* **115**, 2423–2433.
- Gabbita S. P., Lovell M. A. and Markesbery W. R. (1998) Increased nuclear DNA oxidation in the brain in Alzheimer's disease. *J. Neurochem.* **71**, 2034–2040.
- Gaeta A. and Hider R. C. (2005) The crucial role of metal ions in neurodegeneration: the basis for a promising therapeutic strategy. *Br. J. Pharmacol.* **146**, 1041–1059.

- Games D., Adams D., Alessandrini R., *et al.* (1995) Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. *Nature* **373**, 523–527.
- Gibson T. J. and Murphy R. M. (2005) Design of peptidyl compounds that affect beta-amyloid aggregation: importance of surface tension and context. *Biochemistry* **44**, 8898–8907.
- Gilman S., Koller M., Black R. S., *et al.* (2005) Clinical effects of Abeta immunization (AN1792) in patients with AD in an interrupted trial. *Neurology* **64**, 1553–1562.
- Glener G. G. and Wong C. W. (1984) Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem. Biophys. Res. Commun.* **120**, 885–890.
- Good P. F., Werner P., Hsu A., Olanow C. W. and Perl D. P. (1996) Evidence of neuronal oxidative damage in Alzheimer's disease. *Am. J. Pathol* **149**, 21–28.
- Götz J., Chen F., van Dorpe J. and Nitsch R. M. (2001) Formation of neurofibrillary tangles in P3011 tau transgenic mice induced by Abeta 42 fibrils. *Science* **293**, 1491–1495.
- Green P. S., Bales K., Paul S. and Bu G. (2005) Estrogen therapy fails to alter amyloid deposition in the PDAPP model of Alzheimer's disease. *Endocrinology* **146**, 2774–2781.
- Griffin W. S., Stanley L. C., Ling C., White L., MacLeod V., Perrot L. J., White C. L. 3rd and Araoz C. (1989) Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A.* **86**, 7611–7615.
- Gundersen E., Fan K., Haas K., *et al.* (2005) Molecular-modeling based design, synthesis, and activity of substituted piperidines as gamma-secretase inhibitors. *Bioorg. Med. Chem. Lett.* **15**, 1891–1894.
- Hanessian S., Yun H., Hou Y., *et al.* (2005) Structure-based design, synthesis, and memapsin 2 (BACE) inhibitory activity of carbocyclic and heterocyclic peptidomimetics. *J. Med. Chem.* **48**, 5175–5190.
- 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.
- Hardy J., Duff K., Hardy K. G., Perez-Tur J. and Hutton M. (1998) Genetic dissection of Alzheimer's disease and related dementias: amyloid and its relationship to tau. *Nat. Neurosci.* **1**, 355–358.
- Harper J. D. and Lansbury P. T. Jr (1997) Models of amyloid seeding in Alzheimer's disease and scrapie: mechanistic truths and physiological consequences of the time-dependent solubility of amyloid proteins. *Annu. Rev. Biochem.* **66**, 385–407.
- Harper J. D., Wong S. S., Lieber C. M., and Lansbury P. T. Jr (1999) Assembly of A beta amyloid protofibrils: an in vitro model for a possible early event in Alzheimer's disease. *Biochemistry* **38**, 8972–8980.
- Hartman R. E., Izumi Y., Bales K. R., Paul S. M., Wozniak D. F. and Holtzman D. M. (2005) Treatment with an amyloid-beta antibody ameliorates plaque load, learning deficits, and hippocampal long-term potentiation in a mouse model of Alzheimer's disease. *J. Neurosci.* **25**, 6213–6220.
- Hemming M. L. and Selkoe D. J. (2005) Amyloid {beta}-protein is degraded by cellular angiotensin-converting enzyme (ACE) and elevated by an ACE inhibitor. *J. Biol. Chem.* **280**, 37 644–37 650.
- Hennessy E. J. and Buchwald S. L. (2005) Synthesis of 4,5-dianilino-phthalimide and related analogues for potential treatment of Alzheimer's disease via palladium-catalyzed amination. *J. Org. Chem.* **70**, 7371–7375.
- Henry A., Li Q. X., Galatis D., Hesse L., Multhaup G. and Beyreuther K., Masters C. L. and Cappai R. (1998) Inhibition of platelet activation by the Alzheimer's disease amyloid precursor protein. *Br. J. Haematol* **103**, 402–415.
- Higuchi M., Yanai K., Okamura N., *et al.* (2000) Histamine H (1) receptors in patients with Alzheimer's disease assessed by positron emission tomography. *Neuroscience* **99**, 721–729.
- Higuchi M., Iwata N., Matsuba Y., Sato K., Sasamoto K. and Saido T. C. (2005) (19)F and (1)H MRI detection of amyloid beta plaques in vivo. *Nat. Neurosci.* **8**, 527–533.
- Höglund K., Syversen S., Lewczuk P., Wallin A., Wiltfang J. and Blennow K. (2005a) Statin treatment and a disease-specific pattern of beta-amyloid peptides in Alzheimer's disease. *Exp Brain Res.* **164**, 205–214.
- Höglund K., Thelen K. M., Syversen S., *et al.* (2005b) The effect of simvastatin treatment on the amyloid precursor protein and brain cholesterol metabolism in patients with Alzheimer's disease. *Dement. Geriatr. Cogn. Disord.* **19**, 256–265.
- Hsia A. Y., Masliah E., McConlogue L., *et al.* (1999) Plaque-independent disruption of neural circuits in Alzheimer's disease mouse models. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 3228–3233.
- 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 J., LaDu M. J. and Van Eldik L. J. (1998) Apolipoprotein E attenuates beta-amyloid-induced astrocyte activation. *J. Neurochem.* **71**, 1626–1634.
- Huang X., Atwood C. S., Moir R. D., Hartshorn M. A., Vonsattel J. P., Tanzi R. E. and Bush A. I. (1997) Zinc-induced Alzheimer's Abeta1–40 aggregation is mediated by conformational factors. *J. Biol. Chem.* **272**, 26 464–26 470.
- Huang D. Luthi U., Kolb P., Edler K., Cecchini M., Audetat S., Barberis A. and Cafilisch A. (2005) Discovery of cell-permeable non-peptide inhibitors of beta-secretase by high-throughput docking and continuum electrostatics calculations. *J. Med. Chem.* **48**, 5108–5111.
- Hutton M., Lendon C. L., Rizzu P., *et al.* (1998) Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature* **393**, 702–705.
- Hynes T. R., Randal M., Kennedy L. A., Eigenbrot C. and Kossiakoff A. A. (1990) X-ray crystal structure of the protease inhibitor domain of Alzheimer's amyloid beta-protein precursor. *Biochemistry* **29**, 10 018–10 022.
- Ibach B., Haen E., Marienhagen J. and Hajak G. (2005) Clioquinol treatment in familiar early onset of Alzheimer's disease: a case report. *Pharmacopsychiatry* **38**, 178–179.
- Irizarry M. C., Soriano F., McNamara M., Page K. J., Schenk D., Games D. and Hyman B. T. (1997) Abeta deposition is associated with neurophil changes, but not with overt neuronal loss in the human amyloid precursor protein V717F (PDAPP) transgenic mouse. *J. Neurosci.* **17**, 7053–7059.
- Jankowsky J. L., Slunt H. H., Gonzales V., *et al.* (2005) Persistent amyloidosis following suppression of abeta production in a transgenic model of Alzheimer disease. *PLoS Med.* **2**, e355.
- Jantzen P. T., Connor K. E., DiCarlo G., Wenk G. L., Wallace J. L., Rojiani A. M., Coppola D., Morgan D. and Gordon M. N. (2002) Microglial activation and beta-amyloid deposit reduction caused by a nitric oxide-releasing nonsteroidal anti-inflammatory drug in amyloid precursor protein plus presenilin-1 transgenic mice. *J. Neurosci.* **22**, 2246–2254.
- Janus C., Pearson J., McLaurin J., *et al.* (2000) Aβ-peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. *Nature* **408**, 979–982.
- Jessen F., Block W., Traber F., Keller E., Flacke S., Papassotiropoulos A., Lamerichs R., Heun R. and Schild H. H. (2000) Proton MR spectroscopy detects a relative decrease of N-acetylaspartate in the medial temporal lobe of patients with AD. *Neurology* **55**, 684–688.
- Jhamandas J. H., Wie M. B., Harris K., MacTavish D. and Kar S. (2005) Fucooidan inhibits cellular and neurotoxic effects of beta-amyloid (A beta) in rat cholinergic basal forebrain neurons. *Eur. J. Neurosci.* **21**, 2649–2659.

- Jobst K. A., Smith A. D., Szatmari M., *et al.* (1992) Detection in life of confirmed Alzheimer's disease using a simple measurement of medial temporal lobe atrophy by computed tomography. *Lancet* **340**, 1179–1183.
- Jobst K. A., Barnetson L. P. and Shepstone B. J. (1998) Accurate prediction of histologically confirmed Alzheimer's disease and the differential diagnosis of dementia: the use of NINCDS-ADRDA and DSM-III-R. criteria, SPECT, x-ray CT, and Apo E4 in medial temporal lobe dementias: Oxford Project to Investigate Memory and Aging. *Int. Psychogeriatr.* **10**, 271–302.
- Jordan J., Galindo M. F., Miller R. J., Reardon C. A., Getz G. S. and LaDu M. J. (1998) Isoform-specific effect of apolipoprotein E on cell survival and beta-amyloid-induced toxicity in rat hippocampal pyramidal neuronal cultures. *J. Neurosci.* **18**, 195–204.
- Kagan B. L., Hirakura Y., Azimov R., Azimova R. and Lin M. C. (2002) The channel hypothesis of Alzheimer's disease: current status. *Peptides* **23**, 1311–1315.
- Kalaria R. N. (2002) Small vessel disease and Alzheimer's dementia: pathological considerations. *Cerebrovasc. Dis.* **13**, 48–52.
- Kanapathipillai M., Lentzen G., Sierks M. and Park C. B. (2005) Ectoine and hydroxyectoine inhibit aggregation and neurotoxicity of Alzheimer's beta-amyloid. *FEBS Lett.* **579**, 4775–4780.
- Kang J., Lemaire H. G., Unterbeck A., Salbaum J. M., Masters C. L., Grzeschik K. H., Multhaup G., Beyreuther K. and Muller-Hill B. (1987) The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature* **325**, 733–736.
- Kantarci K., Jack C. R., Xu Y. C., *et al.* (2001) Mild cognitive impairment and Alzheimer disease: regional diffusivity of water. *Radiology* **219**, 101–107.
- Kawahara M. (2004) Disruption of calcium homeostasis in the pathogenesis of Alzheimer's disease and other conformational diseases. *Curr. Alzheimer Res.* **1**, 87–95.
- Keller J. N., Pang Z., Geddes J. W., Begley J. G., Germeyer A., Waeg G. and Mattson M. P. (1997) Impairment of glucose and glutamate transport and induction of mitochondrial oxidative stress and dysfunction in synaptosomes by amyloid beta-peptide: role of the lipid peroxidation product 4-hydroxynonenal. *J. Neurochem.* **69**, 273–284.
- Kepe V., Barrio J. R., Huang S. C., *et al.* (2006) Serotonin 1A receptors in the living brain of Alzheimer's disease patients. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 702–707.
- Kerr M. L. and Small D. H. (2005) Cytoplasmic domain of the beta-amyloid protein precursor of Alzheimer's disease: function, regulation of proteolysis, and implications for drug development. *J. Neurosci. Res.* **80**, 151–159.
- Killiany R. J., Hyman B. T., Gomez-Isla T., Moss M. B., Kikinis R., Jolesz F., Tanzi R., Jones K. and Albert M. S. (2002) MRI measures of entorhinal cortex vs hippocampus in preclinical AD. *Neurology* **58**, 1188–1196.
- Kimberly W. T., Xia W., Rahmati T., Wolfe M. S. and Selkoe D. J. (2000) The transmembrane aspartates in presenilin 1 and 2 are obligatory for gamma-secretase activity and amyloid beta-protein generation. *J. Biol. Chem.* **275**, 3173–3178.
- Kimura M., Akasofu S., Ogura H. and Sawada K. (2005) Protective effect of donepezil against Abeta (1–40) neurotoxicity in rat septal neurons. *Brain Res.* **1047**, 72–84.
- Klunk W. E., Wang Y., Huang G. F., *et al.* (2003) The binding of 2-(4'-methylaminophenyl) benzothiazole to postmortem brain homogenates is dominated by the amyloid component. *J. Neurosci.* **23**, 2086–2092.
- Klunk W. E., Engler H., Nordberg A., *et al.* (2004) Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. *Ann. Neurol.* **55**, 306–319.
- Klyubin I., Walsh D. M., Lemere C. A., *et al.* (2005) Amyloid beta protein immunotherapy neutralizes Abeta oligomers that disrupt synaptic plasticity in vivo. *Nat. Med.* **11**, 556–561.
- Kornacker M. G., Lai Z., Witmer M., *et al.* (2005) An inhibitor binding pocket distinct from the catalytic active site on human beta-APP cleaving enzyme. *Biochemistry* **44**, 11 567–11 573.
- Kruman I. I. and Mattson M. P. (1999) Pivotal role of mitochondrial calcium uptake in neural cell apoptosis and necrosis. *J. Neurochem.* **72**, 529–540.
- Lambourne S. L., Sellers L. A., Bush T. G., Choudhury S. K., Emson P. C., Suh Y. H. and Wilkinson L. S. (2005) Increased tau phosphorylation on mitogen-activated protein kinase consensus sites and cognitive decline in transgenic models for Alzheimer's disease and FTDP-17: evidence for distinct molecular processes underlying tau abnormalities. *Mol. Cell Biol.* **25**, 278–293.
- Lanz T. A., Hosley J. D., Adams W. J. and Merchant K. M. (2004) Studies of Abeta pharmacodynamics in the brain, cerebrospinal fluid, and plasma in young (plaque-free) Tg2576 mice using the gamma-secretase inhibitor N2-[(2S)-2-(3,5-difluorophenyl)-2-hydroxyethanoyl]-N1-[(7S)-5-methyl-6-oxo-6,7-dihydro-5H-dibenzo[b,d]azepin-7-yl]-L-alaninamide (LY-411575). *J. Pharmacol. Exp. Ther.* **309**, 49–55.
- Laras Y., Quelever G., Garino C., Pietrancosta N., Sheha M., Bihel F., Wolfe M. S. and Kraus J. L. (2005) Substituted thiazolamide coupled to a redox delivery system: a new gamma-secretase inhibitor with enhanced pharmacokinetic profile. *Org. Biomol. Chem.* **3**, 612–618.
- Law A., Gauthier S. and Quirion R. (2001) Say NO to Alzheimer's disease: the putative links between nitric oxide and dementia of the Alzheimer's type. *Brain Res. Brain Res. Rev.* **35**, 73–96.
- Lecanu L., Yao W., Piechot A., Greeson J., Tzalis D. and Papadopoulos V. (2005) Identification, design, synthesis, and pharmacological activity of (4-ethyl-piperazin-1-yl)-phenylmethanone derivatives with neuroprotective properties against beta-amyloid-induced toxicity. *Neuropharmacology* **49**, 86–96.
- Lee E. B., Leng L. Z., Lee V. M. and Trojanowski J. Q. (2005a) Meningoencephalitis associated with passive immunization of a transgenic murine model of Alzheimer's amyloidosis. *FEBS Lett.* **579**, 2564–2568.
- Lee H. J., Seong Y. H., Bae K. H., *et al.* (2005b) Beta-secretase (BACE1) inhibitors from *Sanguisorba Radix*. *Arch. Pharm. Res.* **28**, 799–803.
- Lee J. Y., Cole T. B., Palmiter R. D., Suh S. W. and Koh J. Y. (2002) Contribution by synaptic zinc to the gender-disparate plaque formation in human Swedish mutant APP transgenic mice. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 7705–7710.
- Lee K. H., Shin B. H., Shin K. J., Kim D. J. and Yu J. (2005c) A hybrid molecule that prohibits amyloid fibrils and alleviates neuronal toxicity induced by beta-amyloid (1–42). *Biochem. Biophys. Res. Commun.* **328**, 816–823.
- Lee M., Bard F., Johnson-Wood K., Lee C., Hu K., Griffith S. G., Black R. S., Schenk D. and Seubert P. (2005d) Abeta42 immunization in Alzheimer's disease generates Abeta N-terminal antibodies. *Ann. Neurol.* **58**, 430–435.
- Lee S., Carson K., Rice-Ficht A. and Good T. (2005e) Hsp20, a novel alpha-crystallin, prevents Abeta fibril formation and toxicity. *Protein Sci.* **14**, 593–601.
- Lee Y., Aono M., Laskowitz D., Warner D. S. and Pearlstein R. D. (2004) Apolipoprotein E protects against oxidative stress in mixed neuronal-glia cell cultures by reducing glutamate toxicity. *Neurochem. Int.* **44**, 107–118.
- Lefranc-Jullien S., Lisowski V., Hernandez J. F., Martinez J. and Checler F. (2005) Design and characterization of a new cell-permeant

- inhibitor of the beta-secretase BACE1. *Br. J. Pharmacol.* **145**, 228–235.
- Lemere C. A., Blusztajn J. K., Yamaguchi H., Wisniewski T., Saido T. C. and Selkoe D. J. (1996) Sequence of deposition of heterogeneous amyloid beta-peptides and APO E in Down syndrome: implications for initial events in amyloid plaque formation. *Neurobiol. Dis.* **3**, 16–32.
- de Leon M. J., DeSanti S., Zinkowski R., *et al.* (2006) Longitudinal CSF and MRI biomarkers improve the diagnosis of mild cognitive impairment. *Neurobiol. Aging* **27**, 394–401.
- Lewis J., Dickson D. W., Lin W. L., *et al.* (2001) Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. *Science* **293**, 1487–1491.
- Lewis S. J., Smith A. L., Neduvilil J. G., *et al.* (2005) A novel series of potent gamma-secretase inhibitors based on a benzobicyclo[4.2.1]nonane core. *Bioorg. Med. Chem. Lett.* **15**, 373–378.
- Lim G. P., Yang F., Chu T., *et al.* (2001) Ibuprofen effects on Alzheimer pathology and open field activity in APPsw transgenic mice. *Neurobiol. Aging* **22**, 983–991.
- Lim G. P., Calon F., Morihara T., Yang F., Teter B., Ubeda O., Salem N., Frautschy S. A. Jr and Cole G. M. (2005) A diet enriched with the omega-3 fatty acid docosahexaenoic acid reduces amyloid burden in an aged Alzheimer mouse model. *J. Neurosci.* **25**, 3032–3040.
- Lippa C. F., Nee L. E., Mori H. and St George-Hyslop P. (1998) Abeta-42 deposition precedes other changes in PS-1 Alzheimer's disease. *Lancet* **352**, 1117–1118.
- Liu G., Garrett M. R., Men P., Zhu X., Perry G. and Smith M. A. (2005) Nanoparticle and other metal chelation therapeutics in Alzheimer disease. *Biochim. Biophys. Acta* **1741**, 246–252.
- Lockhart A., Judd L., Ye D. B., Merritt A. T., Lowe P. N., Morgenstern J. L., Hong G., Gee A. D. and Brown J. (2005) Evidence for the presence of three distinct binding sites for the thioflavin T class of Alzheimer's disease PET imaging agents on beta-amyloid peptide fibrils. *J. Biol. Chem.* **280**, 7677–7684.
- Lovell M. A., Ehmann W. D., Butler S. M. and Markesbery W. R. (1995) Elevated thiobarbituric acid-reactive substances and antioxidant enzyme activity in the brain in Alzheimer's disease. *Neurology* **45**, 1594–1601.
- Lovell M. A., Robertson J. D., Teesdale W. J., Campbell J. L. and Markesbery W. R. (1998) Copper, iron and zinc in Alzheimer's disease senile plaques. *J. Neurol. Sci.* **158**, 47–52.
- Lovestone S. and Reynolds C. H. (1997) The phosphorylation of tau: a critical stage in neurodevelopment and neurodegenerative processes. *Neuroscience* **78**, 309–324.
- Ma Q., Li Y., Du J., Liu H., Kanazawa K., Nemoto T., Nakanishi H. and Zhao Y. (2006) Copper binding properties of a tau peptide associated with Alzheimer's disease studied by CD, NMR, and MALDI-TOF MS. *Peptides* **27**, 841–849.
- Mahdi F., Van Nostrand W. E. and Schmaier A. H. (1995) Protease nexin-2/amyloid beta-protein precursor inhibits factor Xa in the prothrombinase complex. *J. Biol. Chem.* **270**, 23 468–23 474.
- Marambaud P., Zhao H. and Davies P. (2005) Resveratrol promotes clearance of Alzheimer's disease amyloid- $\beta$  peptides. *J. Biol. Chem.* **280**, 37 377–37 382.
- Mark R. J., Hensley K., Butterfield D. A. and Mattson M. P. (1995) Amyloid beta-peptide impairs ion-motive ATPase activities: evidence for a role in loss of neuronal Ca<sup>2+</sup> homeostasis and cell death. *J. Neurosci.* **15**, 6239–6249.
- Mark R. J., Fuson K. S. and May P. C. (1999) Characterization of 8-epiprostaglandin F<sub>2</sub>alpha as a marker of amyloid beta-peptide-induced oxidative damage. *J. Neurochem.* **72**, 1146–1153.
- Marques M. A. and Crutcher K. A. (2003) Apolipoprotein E-related neurotoxicity as a therapeutic target for Alzheimer's disease. *J. Mol. Neurosci.* **20**, 327–337.
- Marrazzo A., Caraci F., Salinaro E. T., Su T. P., Copani A. and Ronsisvalle G. (2005) Neuroprotective effects of sigma-1 receptor agonists against beta-amyloid-induced toxicity. *Neuroreport* **16**, 1223–1226.
- Marshall J. R., Stimson E. R., Ghilardi J. R., Vinters H. V., Mantyh P. W. and Maggio J. E. (2002) Noninvasive imaging of peripherally injected Alzheimer's disease type synthetic A beta amyloid in vivo. *Bioconjug. Chem.* **13**, 276–284.
- Martins R. N., Harper C. G., Stokes G. B. and Masters C. L. (1986) Increased cerebral glucose-6-phosphate dehydrogenase activity in Alzheimer's disease may reflect oxidative stress. *J. Neurochem.* **46**, 1042–1045.
- Masliah E., Hansen L., Adame A., *et al.* (2005) Abeta vaccination effects on plaque pathology in the absence of encephalitis in Alzheimer disease. *Neurology* **64**, 129–131.
- Masse I., Bordet R., Deplanque D., Al Khedr A., Richard F., Libersa C. and Pasquier F. (2005) Lipid lowering agents are associated with a slower cognitive decline in Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry* **76**, 1624–1629.
- Masters C. L. and Beyreuther K. (1998) Alzheimer's disease. *Br. Med. J.* **316**, 446–448.
- Masters C. L. and Beyreuther K. (2005) The neuropathology of Alzheimer's disease in the year 2005, in *Neurodegenerative Diseases: Neurobiology, Pathogenesis and Therapeutics* (Beal M. F., Lang A. E. and Ludolph A. C., eds), pp. 433–440. Cambridge University Press, Cambridge.
- Masters C. L., Simms G., Weinman N. A., Multhaup G., McDonald B. L. and Beyreuther K. (1985) Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4245–4249.
- Mathis C. A., Bacskai B. J., Kajdasz S. T., *et al.* (2002) A lipophilic thioflavin-T derivative for positron emission tomography (PET) imaging of amyloid in brain. *Bioorg. Med. Chem. Lett.* **12**, 295–298.
- Mattson M. P. and Chan S. L. (2003) Neuronal and glial calcium signaling in Alzheimer's disease. *Cell Calcium* **34**, 385–397.
- Mattson M. P., Tomaselli K. J. and Rydel R. E. (1993) Calcium-destabilizing and neurodegenerative effects of aggregated beta-amyloid peptide are attenuated by basic FGF. *Brain Res.* **621**, 35–49.
- Mayeux R., Saunders A. M., Shea S., *et al.* (1998) Utility of the apolipoprotein E genotype in the diagnosis of Alzheimer's disease: Alzheimer's Disease Centers Consortium on Apolipoprotein E and Alzheimer's Disease. *N. Engl. J. Med.* **338**, 506–511.
- Maynard C. J., Cappai R., Volitakis I., Cherny R. A., White A. R., Beyreuther K., Masters C. L., Bush A. I. and Li Q. X. (2002) Overexpression of Alzheimer's disease amyloid-beta opposes the age-dependent elevations of brain copper and iron. *J. Biol. Chem.* **277**, 44 670–44 676.
- McKhann G., Drachman D., Folstein M., Katzman R., Price D. and Stadlan E. M. (1984) Clinical diagnosis of Alzheimer's disease. Report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* **34**, 939–944.
- McLaurin J., Cecal R., Kierstead M. E., *et al.* (2002) Therapeutically effective antibodies against amyloid-beta peptide target amyloid-beta residues 4–10 and inhibit cytotoxicity and fibrillogenesis. *Nat. Med.* **8**, 1263–1269.
- McLean C. A., Cherny R. A., Fraser F. W., Fuller S. J., Smith M. J., Beyreuther K., Bush A. I. and Masters C. L. (1999) Soluble pool of A $\beta$  amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. *Ann. Neurol.* **46**, 860–866.
- Mecocci P., MacGarvey U. and Beal M. F. (1994) Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. *Ann. Neurol.* **36**, 747–751.

- Meda L., Cassatella M. A., Szendrei G. I., Otvos L., Baron P. Jr, Villalba M., Ferrari D. and Rossi F. (1995) Activation of microglial cells by beta-amyloid protein and interferon-gamma. *Nature* **374**, 647–650.
- Mettenberg J. M., Arandjelovic S. and Gonias S. L. (2005) A chemically modified preparation of alpha2-macroglobulin binds beta-amyloid peptide with increased affinity and inhibits Abeta cytotoxicity. *J. Neurochem.* **93**, 53–62.
- Michaelis M. L., Ansar S., Chen Y., Reiff E. R., Seyb K. I., Himes R. H., Audus K. L. and Georg G. I. (2005) {beta}-Amyloid-induced neurodegeneration and protection by structurally diverse microtubule-stabilizing agents. *J. Pharmacol. Exp. Ther.* **312**, 659–668.
- Miklosy J., Taddei K., Suva D., et al. (2003) Two novel presenilin-1 mutations (Y256S and Q222H) are associated with early-onset Alzheimer's disease. *Neurobiol. Aging* **24**, 655–662.
- Miura T., Suzuki K., Kohata N. and Takeuchi H. (2000) Metal binding modes of Alzheimer's amyloid beta-peptide in insoluble aggregates and soluble complexes. *Biochemistry* **39**, 7024–7031.
- Mohanakrishnan P., Fowler A. H., Vonsattel J. P., Husain M. M., Jolles P. R., Liem P. and Komoroski R. A. (1995) An in vitro 1H nuclear magnetic resonance study of the temporoparietal cortex of Alzheimer brains. *Exp. Brain Res.* **102**, 503–510.
- Moir R. D., Atwood C. S., Romano D. M., Laurans M. H., Huang X., Bush A. I., Smith J. D. and Tanzi R. E. (1999) Differential effects of apolipoprotein E isoforms on metal-induced aggregation of A beta using physiological concentrations. *Biochemistry* **38**, 4595–4603.
- Moosmann B. and Behl C. (2002) Antioxidants as treatment for neurodegenerative disorders. *Expert Opin. Invest. Drugs* **11**, 1407–1435.
- Moreira P. I., Santos M. S., Sena C., Nunes E., Seica R. and Oliveira C. R. (2005) CoQ10 therapy attenuates amyloid beta-peptide toxicity in brain mitochondria isolated from aged diabetic rats. *Exp. Neurol.* **196**, 112–119.
- Morihara T., Teter B., Yang F., Lim G. P., Boudinot S., Boudinot F. D., Frautsch S. A. and Cole G. M. (2005) Ibuprofen suppresses interleukin-1beta induction of pro-amyloidogenic alpha1-antichymotrypsin to ameliorate beta-amyloid (Abeta) pathology in Alzheimer's models. *Neuropsychopharmacology* **30**, 1111–1120.
- Morris J. C. and Price A. L. (2001) Pathologic correlates of nondemented aging, mild cognitive impairment, and early-stage Alzheimer's disease. *J. Mol. Neurosci.* **17**, 101–118.
- Mucke L., Masliah E., Yu G. Q., et al. (2000) High-level neuronal expression of beta 1–42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. *J. Neurosci.* **20**, 4050–4058.
- Mudher A. and Lovestone S. (2002) Alzheimer's disease – do tauists and baptists finally shake hands? *Trends Neurosci.* **25**, 22–26.
- Mukrasch M. D., Biernat J., von Bergen M., Griesinger C., Mandelkow E. and Zweckstetter M. (2005) Sites of Tau important for aggregation populate {beta}-structure and bind to microtubules and polyanions. *J. Biol. Chem.* **280**, 24 978–24 986.
- Multhaup G., Schlicksupp A., Hesse L., Behr D., Ruppert T., Masters C. L. and Beyreuther K. (1996) The amyloid precursor protein of Alzheimer's disease in the reduction of copper (II) to copper (I). *Science* **271**, 1406–1409.
- Naslund J., Schierhorn A., Hellman U., et al. (1994) Relative abundance of Alzheimer A beta amyloid peptide variants in Alzheimer disease and normal aging. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 8378–8382.
- Necula M., Chirita C. N. and Kuret J. (2005) Cyanine dye N744 inhibits tau fibrillization by blocking filament extension: implications for the treatment of tauopathic neurodegenerative diseases. *Biochemistry* **44**, 10 227–10 237.
- Noble W., Planel E., Zehr C., et al. (2005) Inhibition of glycogen synthase kinase-3 by lithium correlates with reduced tauopathy and degeneration in vivo. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 6990–6995.
- Nordberg A. (1993) In vivo detection of neurotransmitter changes in Alzheimer's disease. *Ann. N.Y. Acad. Sci.* **695**, 27–33.
- Nordberg A., Amberla K., Shigeta M., et al. (1998) Long-term tacrine treatment in three mild Alzheimer patients: effects on nicotinic receptors, cerebral blood flow, glucose metabolism, EEG, and cognitive abilities. *Alzheimer Dis. Assoc. Disord.* **12**, 228–237.
- Okamura N., Suemoto T., Furumoto S., et al. (2005) Quinoline and benzimidazole derivatives: candidate probes for in vivo imaging of tau pathology in Alzheimer's disease. *J. Neurosci.* **25**, 10 857–10 862.
- Olson J. M., Goddard K. A. and Dudek D. M. (2001) The amyloid precursor protein locus and very-late-onset Alzheimer disease. *Am. J. Hum. Genet.* **69**, 895–899.
- Opazo C., Huang X., Cherny R. A., et al. (2002) Metalloenzyme-like activity of Alzheimer's disease beta-amyloid. Cu-dependent catalytic conversion of dopamine, cholesterol, and biological reducing agents to neurotoxic H(2)O(2). *J. Biol. Chem.* **277**, 40 302–40 308.
- Padmanabhan G., Becue I., Smith J. A. (1989) Clonidine, in: Analytical Profiles of Drug Substances (Florey K, ed), pp. 57–90. Academic Press, New York.
- Pappolla M. A., Omar R. A., Kim K. S. and Robakis N. K. (1992) Immunohistochemical evidence of oxidative [corrected] stress in Alzheimer's disease. *Am. J. Pathol.* **140**, 621–628.
- Parihar M. S. and Hemmani T. (2003) Phenolic antioxidants attenuate hippocampal neuronal cell damage against kainic acid induced excitotoxicity. *J. Biosci.* **28**, 121–128.
- Parker W. D. Jr, Parks J., Filley C. M. and Kleinschmidt-DeMasters B. K. (1994) Electron transport chain defects in Alzheimer's disease brain. *Neurology* **44**, 1090–1096.
- Parks J., Smith K. T. S., Trimmer P. A., Bennett J. P. Jr and Parker W. D. Jr (2001) Neurotoxic Abeta peptides increase oxidative stress in vivo through NMDA-receptor and nitric-oxide-synthase mechanisms, and inhibit complex IV activity and induce a mitochondrial permeability transition in vitro. *J. Neurochem.* **76**, 1050–1056.
- Patel N. V., Gordon M. N., Connor K. E., Good R. A., Engelman R. W., Mason J., Morgan D. G., Morgan T. E. and Finch C. E. (2005) Caloric restriction attenuates Abeta-deposition in Alzheimer transgenic models. *Neurobiol. Aging* **26**, 995–1000.
- Perl D. P. (2000) Neuropathology of Alzheimer's disease and related disorders. *Neurol. Clin.* **18**, 847–864.
- Perry E. K., Tomlinson B. E., Blessed G., Bergmann K., Gibson P. H. and Perry R. H. (1978) Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. *Br. Med. J.* **2**, 1457–1459.
- Petersen R. C., Smith G. E., Waring S. C., Ivnik R. J., Tangalos E. G. and Kokmen E. (1999) Mild cognitive impairment: clinical characterization and outcome. *Arch. Neurol.* **56**, 303–308.
- Petrella J. R., Coleman R. E. and Doraiswamy P. M. (2003) Neuroimaging and early diagnosis of Alzheimer disease: a look to the future. *Radiology* **226**, 315–336.
- Phelps M. E. (2000) PET: the merging of biology and imaging into molecular imaging. *J. Nucl. Med.* **41**, 661–681.
- Phinney A. L., Drisaldi B., Schmidt S. D., et al. (2003) In vivo reduction of amyloid-beta by a mutant copper transporter. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 14 193–14 198.
- Pietrak B. L., Crouthamel M. C., Tugusheva K., et al. (2005) Biochemical and cell-based assays for characterization of BACE-1 inhibitors. *Anal. Biochem.* **342**, 144–151.
- Piggott M. A., Owens J., O'Brien J., et al. (2003) Muscarinic receptors in basal ganglia in dementia with Lewy bodies, Parkinson's disease and Alzheimer's disease. *J. Chem. Neuroanat.* **25**, 161–173.

- Prasad K. N. A. R., Hovland W. C., Cole K. C., Prasad P. and Nahreini J., Edwards-Prasad and C. P. Andreatta (2000) Multiple antioxidants in the prevention and treatment of Alzheimer disease: analysis of biologic rationale. *Clin. Neuropharmacol.* **23**, 2–13.
- Price J. C., Klunk W. E., Lopresti B. J., *et al.* (2005) Kinetic modeling of amyloid binding in humans using PET imaging and Pittsburgh Compound-B. *J. Cereb. Blood Flow Metab.* **25**, 1528–1547.
- Price J. L. and Morris J. C. (1999) Tangles and plaques in nondemented aging and preclinical Alzheimer's disease. *Ann. Neurol.* **45**, 358–368.
- Puglielli L., Friedlich A. L., Setchell K. D., *et al.* (2005) Alzheimer disease beta-amyloid activity mimics cholesterol oxidase. *J. Clin. Invest.* **115**, 2556–2563.
- Qiao H., Koya R. C., Nakagawa K., Tanaka H., Fujita H., Takimoto M. and Kuzumaki N. (2005) Inhibition of Alzheimer's amyloid-beta peptide-induced reduction of mitochondrial membrane potential and neurotoxicity by gelsolin. *Neurobiol. Aging* **26**, 849–855.
- Quélever G., Kachidian P., Melon C., Garino C., Laras Y., Pietrancosta N., Sheha M. and Louis Kraus J. (2005) Enhanced delivery of gamma-secretase inhibitor DAPT into the brain via an ascorbic acid mediated strategy. *Org. Biomol. Chem.* **3**, 2450–2457.
- Quinn J., Kulhanek D., Nowlin J., Jones R., Pratico D., Rokach J. and Stackman R. (2005) Chronic melatonin therapy fails to alter amyloid burden or oxidative damage in old Tg2576 mice: implications for clinical trials. *Brain Res.* **1037**, 209–213.
- Quintanilla R. A., Muñoz F. J., Metcalfe M. J., Hitschfeld M., Olivares G., Godoy J. A. and Inestrosa N. C. (2005) Trolox and 17 $\beta$ -estradiol protect against amyloid  $\beta$ -peptide neurotoxicity by a mechanism that involves modulation of the Wnt signaling pathway. *J. Biol. Chem.* **280**, 11 615–11 625.
- Racke M. M., Boone L. I., Hepburn D. L., *et al.* (2005) Exacerbation of cerebral amyloid angiopathy-associated microhemorrhage in amyloid precursor protein transgenic mice by immunotherapy is dependent on antibody recognition of deposited forms of amyloid  $\beta$ . *J. Neurosci.* **25**, 629–636.
- Raman B., Ban T., Yamaguchi K. I., Sakai M., Kawai T., Naiki H. and Goto Y. (2005) Metal ion-dependent effects of clioquinol on the fibril growth of an amyloid beta peptide. *J. Biol. Chem.* **280**, 16 157–16 162.
- Ramassamy C., Averill D., Beffert U., *et al.* (2000) Oxidative insults are associated with apolipoprotein E genotype in Alzheimer's disease brain. *Neurobiol. Dis.* **7**, 23–37.
- Ramassamy C., Krzywkowski P., Averill D., Lussier-Cacan S., Theroux L., Christen Y., Davignon J. and Poirier J. (2001) Impact of apoE deficiency on oxidative insults and antioxidant levels in the brain. *Brain Res. Mol. Brain Res.* **86**, 76–83.
- Ramírez B. G., Blázquez C., Gómez del Pulgar T., Guzmán M. and de Ceballos M. L. (2005) Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. *J. Neurosci.* **25**, 1904–1913.
- Rapoport M., Dawson H. N., Binder L. I., Vitek M. P. and Ferreira A. (2002) Tau is essential to beta-amyloid-induced neurotoxicity. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 6364–6369.
- Refolo L. M., Malester B., LaFrancois J., Bryant-Thomas T., Wang R., Tint G. S., Sambamurti K., Duff K. and Pappolla M. A. (2000) Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model. *Neurobiol. Dis.* **7**, 321–331.
- Refolo L. M., Pappolla M. A., LaFrancois J., *et al.* (2001) A cholesterol-lowering drug reduces beta-amyloid pathology in a transgenic mouse model of Alzheimer's disease. *Neurobiol. Dis.* **8**, 890–899.
- Reisberg B., Doody R., Stoffler A., Schmitt F., Ferris S. and Mobius H. J. (2003) Memantine in moderate-to-severe Alzheimer's disease. *N. Engl. J. Med.* **348**, 1333–1341.
- Rezaei-Zadeh K., Shytle D., Sun N., *et al.* (2005) Green tea epigallocatechin-3-gallate (EGCG) modulates amyloid precursor protein cleavage and reduces cerebral amyloidosis in Alzheimer transgenic mice. *J. Neurosci.* **25**, 8807–8814.
- Ritchie C. W., Bush A. I., Mackinnon A., *et al.* (2003) Metal-protein attenuation with iodochlorhydroxyquin (clioquinol) targeting Abeta amyloid deposition and toxicity in Alzheimer disease: a pilot phase 2 clinical trial. *Arch. Neurol.* **60**, 1685–1691.
- Rodriguez-Martin T., Garcia-Blanco M. A., Mansfield S. G., Grover A. C., Hutton M., Zhou Q., Yu J., Anderton B. H. and Gallo J. M. (2005) Reprogramming of tau alternative splicing by spliceosome-mediated RNA trans-splicing: implications for tauopathies. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 15 659–15 664.
- Rogaev E. I., Sherrington R., Rogaeva E. A., *et al.* (1995) Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. *Nature* **376**, 775–778.
- Rogawski M. A. and Wenk G. L. (2003) The neuropharmacological basis for the use of memantine in the treatment of Alzheimer's disease. *CNS Drug Rev.* **9**, 275–308.
- Rogers J., Schultz J., Brachova L., Lue L. F., Webster S., Bradt B., Cooper N. R. and Moss D. E. (1992) Complement activation and beta-amyloid-mediated neurotoxicity in Alzheimer's disease. *Res. Immunol.* **143**, 624–630.
- Roher A. E., Chaney M. O., Kuo Y. M., *et al.* (1996) Morphology and toxicity of Abeta-(1–42) dimer derived from neuritic and vascular amyloid deposits of Alzheimer's disease. *J. Biol. Chem.* **271**, 20 631–20 635.
- Rose S. E., Chen F., Chalk J. B., Zelaya F. O., Strugnell W. E., Benson M., Semple J. and Doddrell D. M. (2000) Loss of connectivity in Alzheimer's disease: an evaluation of white matter tract integrity with colour coded MR diffusion tensor imaging. *J. Neurol. Neurosurg. Psychiatry* **69**, 528–530.
- Rossjohn J., Cappai R., Feil S. C., *et al.* (1999) Crystal structure of the N-terminal, growth factor-like domain of Alzheimer amyloid precursor protein. *Nat. Struct. Biol.* **6**, 327–331.
- Rovelet-Lecrux A., Hannequin D., Raux G., *et al.* (2006) APP locus duplication causes autosomal dominant early-onset Alzheimer disease with cerebral amyloid angiopathy. *Nat. Genet.* **38**, 24–26.
- Sair H. I., Doraiswamy P. M. and Petrella J. R. (2004) In vivo amyloid imaging in Alzheimer's disease. *Neuroradiology* **46**, 93–104.
- Saito T., Iwata N., Tsubuki S., Takaki Y., Takano J., Huang S. M., Suemoto T., Higuchi M. and Saido T. C. (2005) Somatostatin regulates brain amyloid beta peptide A $\beta$ (42) through modulation of proteolytic degradation. *Nat. Med.* **11**, 434–439.
- Sakaue F., Saito T., Sato Y., Asada A., Ishiguro K., Hasegawa M. and Hisanaga S. (2005) Phosphorylation of FTDP-17 mutant tau by cyclin-dependent kinase 5 complexed with p35, 25, or p39. *J. Biol. Chem.* **280**, 31 522–31 529.
- Sano M., Ernesto C., Thomas R. G., *et al.* (1997) A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. The Alzheimer's Disease Cooperative Study. *N. Engl. J. Med.* **336**, 1216–1222.
- Santacruz K., Lewis J., Spire T., *et al.* (2005) Tau suppression in a neurodegenerative mouse model improves memory function. *Science* **309**, 476–481.
- Santa-María I., Hernández F., Smith M. A., Perry G., Avila J. and Moreno F. J. (2005) Neurotoxic dopamine quinone facilitates the assembly of tau into fibrillar polymers. *Mol. Cell Biochem.* **278**, 203–212.
- Satlin A., Bodick N., Offen W. W. and Renshaw P. F. (1997) Brain proton magnetic resonance spectroscopy (H-MRS) in Alzheimer's disease: changes after treatment with xanomeline, an M1 selective cholinergic agonist. *Am. J. Psychiatry* **154**, 1459–1461.

- Sato K., Higuchi M., Iwata N., Saido T. C. and Sasamoto K. (2004) Fluoro-substituted and <sup>13</sup>C-labeled styrylbenzene derivatives for detecting brain amyloid plaques. *Eur. J. Med. Chem.* **39**, 573–578.
- Sato T., Tanimura Y., Hirofani N., Saido T. C., Morishima-Kawashima M. and Ihara Y. (2005) Blocking the cleavage at midportion between gamma- and epsilon-sites remarkably suppresses the generation of amyloid beta-protein. *FEBS Lett.* **579**, 2907–2912.
- Saunders A. M., Strittmatter W. J., Schmechel D., et al. (1993) Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology* **43**, 1467–1472.
- Saura C. A., Chen G., Malkani S., et al. (2005) Conditional inactivation of presenilin 1 prevents amyloid accumulation and temporarily rescues contextual and spatial working memory impairments in amyloid precursor protein transgenic mice. *J. Neurosci.* **25**, 6755–6764.
- Sayre L. M., Perry G., Harris P. L., Liu Y., Schubert K. A. and Smith M. A. (2000) In situ oxidative catalysis by neurofibrillary tangles and senile plaques in Alzheimer's disease: a central role for bound transition metals. *J. Neurochem.* **74**, 270–279.
- Scheltens P. H. (2001) Structural neuroimaging of Alzheimer's disease and other dementias. *Aging* **13**, 203–209.
- Schenk D. (2002) Amyloid-beta immunotherapy for Alzheimer's disease: the end of the beginning. *Nat Rev. Neurosci.* **3**, 824–828.
- Schenk D., Hagen M. and Seubert P. (2004) Current progress in beta-amyloid immunotherapy. *Curr. Opin. Immunol.* **16**, 599–606.
- Scherzer C. R., Offe K., Gearing M., et al. (2004) Loss of apolipoprotein E receptor LR11 in Alzheimer disease. *Arch. Neurol.* **61**, 1200–1205.
- Scheuner D., Eckman C., Jensen M., et al. (1996) Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat. Med.* **2**, 864–870.
- Schipling S., Kontush A., Arlt S., Buhmann C., Sturenburg H. J., Mann U., Muller-Thomsen T. and Beisiegel U. (2000) Increased lipoprotein oxidation in Alzheimer's disease. *Free Radic. Biol. Med.* **28**, 351–360.
- Schuff N., Capizzano A. A., Du A. T., et al. (2002) Selective reduction of N-acetylaspartate in medial temporal and parietal lobes in AD. *Neurology* **58**, 928–935.
- Schuster D., Rajendran A., Hui S. W., Nicotera T., Srikrishnan T. and Kruzel M. L. (2005) Protective effect of colostrinin on neuroblastoma cell survival is due to reduced aggregation of beta-amyloid. *Neuropeptides* **39**, 419–426.
- Selkoe D. J. (2001) Alzheimer's disease: genes, proteins, and therapy. *Physiol. Rev.* **81**, 741–766.
- Selkoe D. J. (2002) Alzheimer's disease is a synaptic failure. *Science* **298**, 789–791.
- Selley M. L., Close D. R. and Stern S. E. (2002) The effect of increased concentrations of homocysteine on the concentration of (E)-4-hydroxy-2-nonenal in the plasma and cerebrospinal fluid of patients with Alzheimer's disease. *Neurobiol. Aging* **23**, 383–388.
- Seubert P., Vigo-Pelfrey C., Esch F., et al. (1992) Isolation and quantification of soluble Alzheimer's beta-peptide from biological fluids. *Nature* **359**, 325–327.
- Seubert P., Oltersdorf T., Lee M. G., et al. (1993) Secretion of beta-amyloid precursor protein cleaved at the amino terminus of the beta-amyloid peptide. *Nature* **361**, 260–263.
- Sherrington R., Rogaeve E. I., Liang Y., et al. (1995) Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature* **375**, 754–760.
- Shi J., Perry G., Berridge M. S., Aliev G., Siedlak S. L., Smith M. A., LaManna J. C. and Friedland R. P. (2002) Labeling of cerebral amyloid beta deposits in vivo using intranasal basic fibroblast growth factor and serum amyloid P component in mice. *J. Nucl. Med.* **43**, 1044–1051.
- Shie F. S. M., Montine K. S., Breyer R. M. and Montine T. J. (2005) Microglial EP2 as a new target to increase amyloid beta phagocytosis and decrease amyloid beta-induced damage to neurons. *Brain Pathol.* **15**, 134–138.
- Shimadzu H., Suemoto T., Suzuki M., Shiomitsu T., Okamura N., Kudo Y. and Sawada T. (2003) A novel probe for imaging amyloid- $\beta$ : Synthesis of F-18 labelled BF-108, an Acridine Orange analog. *J. Labelled Comp. Radiopharm.* **46**, 765–772.
- Shoghi-Jadid K., Small G. W., Agdeppa E. D., et al. (2002) Localisation of neurofibrillary tangles and  $\beta$ -amyloid plaques in the brains of living patients with Alzheimer's disease. *Am. J. Geriatr. Psychiatry* **10**, 24–35.
- Si M. L., Long C., Yang D. I., Chen M. F. and Lee T. J. (2005) Statins prevent beta-amyloid inhibition of sympathetic alpha7-nAChR-mediated nitrenergic neurogenic dilation in porcine basilar arteries. *J. Cereb. Blood Flow Metab.* **25**, 1573–1585.
- 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.
- Siemers E. R., Quinn J. F., Kaye J., et al. (2006) Effects of a gamma-secretase inhibitor in a randomized study of patients with Alzheimer disease. *Neurology* **66**, 602–604.
- Silverman D. H. and Phelps M. E. (2001) Application of positron emission tomography for evaluation of metabolism and blood flow in human brain: normal development, aging, dementia, and stroke. *Mol. Genet. Metab.* **74**, 128–138.
- Silverman D. H., Small G. W., Chang C. Y., et al. (2001) Positron emission tomography in evaluation of dementia: regional brain metabolism and long-term outcome. *JAMA* **286**, 2120–2127.
- Singer O., Marr R. A., Rockenstein E., Crews L., Coufal N. G., Gage F. H., Verma I. M. and Masliah E. (2005) Targeting BACE1 with siRNAs ameliorates Alzheimer disease neuropathology in a transgenic model. *Nat. Neurosci.* **8**, 1343–1349.
- Small D. H., Nurcombe V., Reed G., Clarris H., Moir R. and Beyreuther K. and Masters C. L. (1994) A heparin-binding domain in the amyloid protein precursor of Alzheimer's disease is involved in the regulation of neurite outgrowth. *J. Neurosci.* **14**, 2117–2127.
- Smith D. P., Smith D. G., Curtain C. C., et al. (2006) Copper mediated amyloid-beta toxicity is associated with an intermolecular histidine bridge. *J Biol Chem.* [Epub ahead of print].
- Smith M. A., Sayre L. M., Vitek M. P., Monnier V. M. and Perry G. (1995) Early AGEing and Alzheimer's. *Nature* **374**, 316.
- Smith M. A., Harris P. L., Sayre L. M. and Perry G. (1997) Iron accumulation in Alzheimer disease is a source of redox-generated free radicals. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 9866–9868.
- Smith M. J., Kwok J. B., McLean C. A., et al. (2001) Variable phenotype of Alzheimer's disease with spastic paraparesis. *Ann. Neurol.* **49**, 125–129.
- Snyder E. M., Nong Y., Almeida C. G., et al. (2005) Regulation of NMDA receptor trafficking by amyloid-beta. *Nat. Neurosci.* **8**, 1051–1058.
- Spillantini M. G., Bird T. D. and Ghetti B. (1998) Frontotemporal dementia and Parkinsonism linked to chromosome 17: a new group of tauopathies. *Brain Pathol.* **8**, 387–402.
- Spina M. B., Squinto S. P., Miller J., Lindsay R. M. and Hyman C. (1992) Brain-derived neurotrophic factor protects dopamine neurons against 6-hydroxydopamine and N-methyl-4-phenylpyridinium ion toxicity: involvement of the glutathione system. *J. Neurochem.* **59**, 99–106.

- St George-Hyslop P. H. (2000) Genetic factors in the genesis of Alzheimer's disease. *Ann. N.Y. Acad. Sci.* **924**, 1–7.
- St George-Hyslop P., McLachlan D. C., Tsuda T., Rogae E., Karlinsky H., Lippa C. F., Pollen D. and Tuda T. (1994) Alzheimer's disease and possible gene interaction. *Science* **263**, 537.
- Strittmatter W. J., Saunders A. M., Schmechel D., Pericak-Vance M., Enghild J., Salvesen G. S. and Roses A. D. (1993) Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 1977–1981.
- Sultana R., Ravagna A., Mohammad-Abdul H., Calabrese V. and Butterfield D. A. (2005) Ferulic acid ethyl ester protects neurons against amyloid beta-peptide (1–42)-induced oxidative stress and neurotoxicity: relationship to antioxidant activity. *J. Neurochem.* **92**, 749–758.
- Sunderland T., Linker G., Mirza N., *et al.* (2003) Decreased beta-amyloid1–42 and increased tau levels in cerebrospinal fluid of patients with Alzheimer disease. *JAMA* **289**, 2094–2103.
- Swerdlow R. H., Parks J. K., Cassarino D. S., Maguire D. J., Maguire R. S., Bennett J. P. Jr, Davis R. E. and Parker W. D. Jr (1997) Cybrids in Alzheimer's disease: a cellular model of the disease? *Neurology* **49**, 918–925.
- Takahashi S., Takahashi I., Sato H., Kubota Y., Yoshida S. and Muramatsu Y. (2001) Age-related changes in the concentrations of major and trace elements in the brain of rats and mice. *Biol. Trace Elem. Res.* **80**, 145–158.
- Tamagno E., Robino G., Obbili A., Bardini P., Aragno M., Parola M. and Danni O. (2003) H<sub>2</sub>O<sub>2</sub> and 4-hydroxynonenal mediate amyloid beta-induced neuronal apoptosis by activating JNKs and p38MAPK. *Exp. Neurol.* **180**, 144–155.
- Tang B. L. (2005) Alzheimer's disease: channeling APP to non-amyloidogenic processing. *Biochem. Biophys. Res. Commun.* **331**, 375–378.
- Taniguchi S., Suzuki N., Masuda M., Hisanaga S., Iwatsubo T., Goedert M. and Hasegawa M. (2005) Inhibition of heparin-induced tau filament formation by phenothiazines, polyphenols, and porphyrins. *J. Biol. Chem.* **280**, 7614–7623.
- Terry R. D., Masliah E. and Hansen L. A. (1994) Structural basis of the cognitive alterations, in Alzheimer Disease (Terry R. D., Katzman R. and Bick K. L., eds). Raven Press, New York.
- Thompson P. M., Hayashi K. M., De Zubicaray G. L., *et al.* (2004) Mapping hippocampal and ventricular change in Alzheimer disease. *Neuroimage* **22**, 1754–1766.
- Van Dam D., Abramowski D., Staufenbiel M. and De Deyn P. P. (2005) Symptomatic effect of donepezil, rivastigmine, galantamine and memantine on cognitive deficits in the APP23 model. *Psychopharmacology (Berl)* **180**, 177–190.
- Vanhoutte G., Dewachter I., Borghgraef P., Van Leuven F. and Van der Linden A. (2005) Noninvasive in vivo MRI detection of neuritic plaques associated with iron in APP[V717I] transgenic mice, a model for Alzheimer's disease. *Magn. Reson. Med.* **53**, 607–613.
- Villemagne V. L., Ackermann U., Gong S. J., *et al.* (2005a) Abeta amyloid imaging in dementia with Lewy bodies and Alzheimer's disease with 11C-PIB PET. *J. Nucl. Med.* **46**, 124P.
- Villemagne V. L., Rowe C. C., Macfarlane S. and Novakovic K. and Masters C. L. (2005b) *Imaginem Oblivionis*: The prospects of neuroimaging for early detection of Alzheimer's disease. *J. Clin. Neurosci.* **12**, 221–230.
- Wadghiri Y. Z., Sigurdsson E. M., Wisniewski T. and Turnbull D. H. (2005) Magnetic resonance imaging of amyloid plaques in transgenic mice. *Meth Mol Biol.* **299**, 365–379.
- Walker L. C., Price D. L., Voytko M. L. and Schenk D. B. (1994) Labelling of cerebral amyloid in vivo with a monoclonal antibody. *J. Neuropathol. Exp. Neurol.* **53**, 377–383.
- Walker Z., Costa D. C., Walker R. W., *et al.* (2002) Differentiation of dementia with Lewy bodies from Alzheimer's disease using a dopaminergic presynaptic ligand. *J. Neurol. Neurosurg. Psychiatry* **73**, 134–140.
- Walsh D. M., Klyubin I., Fadeeva J. V., Cullen W. K., Anwyl R., Wolfe M. S., Rowan M. J. and Selkoe D. J. (2002) Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* **416**, 535–539.
- Wang J., Ho L., Qin W., *et al.* (2005) Caloric restriction attenuates beta-amyloid neuropathology in a mouse model of Alzheimer's disease. *FASEB J.* **19**, 659–661.
- Wang Y. and Ha Y. (2004) The X-ray structure of an antiparallel dimer of the human amyloid precursor protein E2 domain. *Mol. Cell* **15**, 343–353.
- Weidemann A., Konig G., Bunke D., Fischer P., Salbaum J. M., Masters C. L. and Beyreuther K. (1989) Identification, biogenesis, and localization of precursors of Alzheimer's disease A4 amyloid protein. *Cell* **57**, 115–126.
- Weiner H. L., Lemere C. A., Maron R., Spooner E. T., Grenfell T. J., Mori C., Issazadeh S., Hancock W. W. and Selkoe D. J. (2000) Nasal administration of amyloid- $\beta$  peptide decreases cerebral amyloid burden in a mouse model of Alzheimer's disease. *Ann. Neurol.* **48**, 567–579.
- White A. R., Reyes R., Mercer J. F., *et al.* (1999) Copper levels are increased in the cerebral cortex and liver of APP and APLP2 knockout mice. *Brain Res.* **842**, 439–444.
- Wilcock D. M., DiCarlo G., Henderson D., Jackson J., Clarke K., Ugen K. E., Gordon M. N. and Morgan D. (2003) Intracranially administered anti-A $\beta$  antibodies reduce beta-amyloid deposition by mechanisms both independent of and associated with microglial activation. *J. Neurosci.* **23**, 3745–3751.
- Wolfe M. S. (2002) Therapeutic strategies for Alzheimer's disease. *Nat. Rev. Drug Discov.* **1**, 859–866.
- Wolozin B., Kellman W., Ruosseau P., Celesia G. G. and Siegel G. (2000) Decreased prevalence of Alzheimer disease associated with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. *Arch. Neurol.* **57**, 1439–1443.
- Woltjer R. L., Nghiem W., Maezawa I., Milatovic D., Vaisar T., Montine K. S. and Montine T. J. (2005) Role of glutathione in intracellular amyloid-alpha precursor protein/carboxy-terminal fragment aggregation and associated cytotoxicity. *J. Neurochem.* **93**, 1047–1056.
- Wong G. T., Manfra D., Poulet F. M., *et al.* (2004) Chronic treatment with the gamma-secretase inhibitor LY-411,575 inhibits beta-amyloid peptide production and alters lymphopoiesis and intestinal cell differentiation. *J. Biol. Chem.* **279**, 12 876–12 882.
- Xia W. (2003) Amyloid inhibitors and Alzheimer's disease. *Curr. Opin. Invest. Drugs* **4**, 55–59.
- Xie J. and Guo Q. (2005) PAR-4 is involved in regulation of beta-secretase cleavage of the Alzheimer amyloid precursor protein. *J. Biol. Chem.* **280**, 13 824–13 832.
- Xie Z., Romano D. M. and Tanzi R. E. (2005) Effects of RNAi-mediated silencing of PEN-2, APH-1a, and nicastrin on wild-type vs FAD mutant forms of presenilin 1. *J. Mol. Neurosci.* **25**, 67–78.
- Xu H., Gouras G. K., Greenfield J. P., *et al.* (1998) Estrogen reduces neuronal generation of Alzheimer beta-amyloid peptides. *Nat. Med.* **4**, 447–451.
- Xu H., Faber C., Uchiki T., Racca J. and Dealwis C. (2006) Structures of eukaryotic ribonucleotide reductase I define gemcitabine diphosphate binding and subunit assembly. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 4028–4033.
- Xu Y., Jack C. R. J., O'Brien P. C., Kokmen E., Smith G. E., Ivnik R. J., Boeve B. F., Tangalos R. G. and Petersen R. C. (2000) Usefulness

- of MRI measures of entorhinal cortex versus hippocampus in AD. *Neurology* **54**, 1760–1767.
- Yamada K. T. M., Kamei H., Nagai T., Dohniwa M., Kobayashi K., Yoshida S., Ohhara T., Takauma K. and Nabeshima T. (2005) Effects of memantine and donepezil on amyloid beta-induced memory impairment in a delayed-matching to position task in rats. *Behav. Brain Res.* **162**, 191–199.
- Yamamoto K., Ishikawa T., Sakabe T., Taguchi T., Kawai S. and Marsala M. (1998) The hydroxyl radical scavenger Nicaraven inhibits glutamate release after spinal injury in rats. *Neuroreport* **9**, 1655–1659.
- Yamamoto N., Yokoseki T., Shibata M., Yamaguchi H. and Yanagisawa K. (2005) Suppression of A $\beta$  deposition in brain by peripheral administration of Fab fragments of anti-seed antibody. *Biochem. Biophys. Res. Commun.* **335**, 45–47.
- Yang F., Lim G. P., Begum A. N., *et al.* (2005) Curcumin inhibits formation of amyloid  $\beta$  oligomers and fibrils, binds plaques, and reduces amyloid in vivo. *J. Biol. Chem.* **280**, 5892–5901.
- Yang S. P., Kwon B. O., Gho Y. S. and Chae C. B. (2005) Specific interaction of VEGF165 with beta-amyloid, and its protective effect on beta-amyloid-induced neurotoxicity. *J. Neurochem.* **93**, 118–127.
- Ye L., Morgenstern J. L., Gee A. D., Hong G., Brown J. and Lockhart A. (2005) Delineation of positron emission tomography imaging agent binding sites on beta-amyloid peptide fibrils. *J. Biol. Chem.* **280**, 23 599–23 604.
- Yoshiike Y., Tanemura K., Murayama O., Akagi T., Murayama M., Sato S., Sun X., Tanaka N. and Takashima A. (2001) New insights on how metals disrupt amyloid beta-aggregation and their effects on amyloid-beta cytotoxicity. *J. Biol. Chem.* **276**, 32 293–32 299.
- Youm J. W., Kim H., Han J. H., *et al.* (2005) Transgenic potato expressing Abeta reduce Abeta burden in Alzheimer's disease mouse model. *FEBS Lett.* **579**, 6737–6744.
- Yue X., Lu M., Lancaster T., *et al.* (2005) Brain estrogen deficiency accelerates A $\beta$  plaque formation in an Alzheimer's disease animal model. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 19 198–19 203.
- Zhang J., Yarowsky P., Gordon M. N., Di Carlo G., Munireddy S., van Zijl P. C. and Mori S. (2004) Detection of amyloid plaques in mouse models of Alzheimer's disease by magnetic resonance imaging. *Magn. Reson. Med.* **51**, 452–457.
- Zhang W., Oya S., Kung M. P., Hou C., Maier D. L. and Kung H. F. (2005) F-18 polyethyleneglycol stilbenes as PET imaging agents targeting Abeta aggregates in the brain. *Nucl. Med. Biol.* **32**, 799–809.
- Zimmermann M., Gardoni F., Marcello E., Colciaghi F., Borroni B., Padovani A., Cattabeni F. and Di Luca M. (2004) Acetylcholinesterase inhibitors increase ADAM10 activity by promoting its trafficking in neuroblastoma cell lines. *J. Neurochem.* **90**, 1489–1499.
- Zimmermann M., Borroni B., Cattabeni F., Padovani A. and Di Luca M. (2005) Cholinesterase inhibitors influence APP metabolism in Alzheimer disease patients. *Neurobiol. Dis.* **19**, 237–242.