Research Signpost
37/661 (2), Fort P.O., Trivandrum-695 023, Kerala, India

Advancements in Neurological Research, 2008: 141-177 ISBN: 978-81-308-0225-1
Editor: John H. Zhang

6 Brain injury as a risk factor for Alzheimer's disease: Therapeutic implications

Richard E. Hartman
Department of Psychology, School of Science and Technology, Loma Linda University, Loma Linda, CA, USA

Abstract

The focus of this chapter is brain injury as a risk factor for Alzheimer's disease (AD). The cause of late-onset AD is currently unknown, but most likely has something to do with the buildup of amyloid-beta (Aβ) in the brain. Evidence suggests that brain injury may increase the risk for AD. People with traumatic brain injury, seizures or stroke are more likely to develop Aβ plaques in their brains. Even children with traumatic brain injury may quickly develop AD-like plaques. The most common cause of brain injury in the elderly is stroke, and most AD brains exhibit stroke pathology at autopsy. Animal experiments suggest that

Correspondence/Reprint request: Dr. Richard E. Hartman, Department of Psychology, 11130 Anderson St. 119, Loma Linda CA 92354, USA. E-mail: behavioralneuroscience@gmail.com

brain damage elevates Aβ levels and accelerates plaque deposition. It is possible that brain damage initiates or accelerates AD pathology by causing elevated brain Aβ levels. Potential mechanisms, including upregulated amyloid precursor protein and ApoE, are reviewed. Thus, brain damage may trigger a cascade of events leading to AD. Because AD pathology includes buildup of vascular Aβ, and because this process increases the risk of future stroke, the process may self-propagate. If early efforts are made to prevent excessive Aβ deposition after brain damage such as stroke, it may be possible to delay the onset of AD. Because AD is a disease of the elderly, delaying the onset by only a few years would significantly decrease its prevalence. Therapeutic strategies could include removal of Aβ via antibodies, inhibiting Aβ formation, and nutritional prevention of brain damage with foods rich in polyphenols and/or essential fatty acids.

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder of aging and cause of dementia, affecting approximately 1 in 10 individuals over the age of 65, and 1 in 2 over 85. Memory loss for recent, but not distant, events is the first and most prominent symptom, followed by a progressive decline in cognitive (e.g., language, executive) and motor abilities. Neuropathological hallmarks of the disease include the accumulation of protein deposits ("plaques") between and surrounding the brain's neurons and neurofibrillary tangles (NFTs) inside the neurons. Although the assessment of behavioral symptoms (especially memory loss) can provide a reasonably accurate diagnosis of AD, other forms of dementia can cause similar deficits. Therefore, a diagnosis of AD can only be confirmed after post-mortem identification of these hallmark neuropathological characteristics. Additionally, vascular plaque deposition, mitochondrial dysfunction, inflammation, astrogliosis, microglial activation, and neuronal cell damage and death are observed in the brains of AD patients.

Similar to other age-related neurodegenerative diseases (like Parkinson's disease), the behavioral symptoms of AD are generally not observed until significant levels of neuropathology and neurodegeneration have accumulated. Because this process may take as long as 20 years[1-5], post-mortem examination of brains from elderly, but cognitively normal, people often reveals both the plaques and tangles indicative of AD. The concept of cognitive reserve, which suggests that certain people may be more (or less) susceptible to the behavioral impact of neurotoxicity and/or neurodegeneration than others[6-8], may explain this phenomenon.

The plaques that build up in the brain are composed predominantly of the amyloid-β (Aβ) peptide[9], but also contain other proteins (e.g., apolipoproteins) and non-proteins (e.g., hemes[10,11], metals). Deposition generally starts in the

medial temporal lobes, primarily within and around the entorhinal cortex and hippocampal formation, an area of the brain important for learning and memory, and then gradually spreads throughout the cortical and subcortical areas[12]. Neurons engulfed by the plaques have abnormal and twisted neuronal processes (axons and dendrites) associated with intracellular NFTs. The NFTs are caused by intracellular aggregates of tau, a microtubule-associated protein that normally helps to stabilize the cytoskeleton of the long neuronal processes. These aggregates (paired helical filaments) of tau eventually damage the cytoskeletal microtubules, leading to the disruption of intracellular transport mechanisms, formation of NFTs, and dystrophic neurites. The plaques are also associated with inflammation, microglial activation, synaptic loss, neuronal atrophy and apoptotic cell death, suggesting that the accumulation of Aβ is somehow neurotoxic. Levels of neurotransmitters in the brain, including acetylcholine (ACh), decrease as the neurons that produce these neurotransmitters atrophy and die[13].

Aβ toxicity

The nature of Aβ's toxicity remains elusive, but probably involves several inter-related mechanisms. *In vivo* and *in vitro* studies imply that Aβ induces damage via oxidative stress. For example, intracellular Aβ can enter cellular mitochondria, inducing free radical formation and inflammation[14,15]. Furthermore, extracellular Aβ-heme complexes that form within plaques can cause oxidative damage to muscarinic ACh receptors, which is prevented by treatment with antioxidant compounds[10,11,16]. Additionally**,** Aβ deposits are associated with intracellular tau disruption and neuronal death in hippocampal cell cultures[17], and may also induce hypersensitivity to excitotoxic neuronal damage[18]. Whatever the mechanism, locally elevated concentrations of Aβ seem to cause a loss of proper neuronal function as measured by long-term potentiation (LTP)[19-22]. The amount of synaptic loss within Aβ plaques currently provides a better biomarker of cognitive dysfunction than amount of plaque deposition *per se* [23-26].

It is also unclear as to which form of Aβ is toxic. At high enough concentrations, soluble monomeric Aβ proteins start to polymerize. Dense-core aggregates of Aβ plaques with a β-sheet (amyloid) conformation, but not diffusely aggregated Aβ, are generally associated with dystrophic neuronal processes[27,28]. Other studies suggest that toxicity results from particular intermediate species of Aβ aggregates (e.g., oligomeric) rather than deposited Aβ plaques [24,29-31].

APP and Aβ production

The Aβ peptide consists of 39-43 amino acids, and is normally enzymatically cleaved from the much larger amyloid precursor protein (APP).

The physiological roles of Aβ, if any, are unknown. The physiological roles of APP, which is a transmembrane protein with a large extracellular domain, may involve neuronal development and/or synaptic plasticity[32]. APP is axonally transported (and plays a role in axonal transport) and accumulates in presynaptic terminals and growth cones[33]**.** It may function to inhibit synapse formation, as *in vitro* cultures of $APP^{-/-}$ hippocampal neurons (which lack APP) have a higher synaptic density[33]. An overabundance of synapses may partially explain the heightened sensitivity to kainic acid-induced excitotoxic seizures[34] and memory deficits observed in $APP^{-/-}$ mice[35]. Mice that lack APP and the APP-like proteins APLP1 and APLP2 (similar proteins that lack the internal Aβ sequence) die soon after birth with marked neuroanatomical and behavioral abnormalities[36]. Thus, APP may share some redundant physiological functions with the APLPs.

Full length APP in the brain is metabolized into a number of fragments by the alpha- (α), beta- (β), and gamma- (γ) secretases, yielding a variety of amino- (N-) terminal, internal, and carboxy- (C-) terminal peptides. In general, APP cleavage tends to follow one of two pathways. Aβ is produced by the so-called amyloidogenic pathway, and prevented by the non-amyloidogenic pathway[37,38].

APP processing – The amyloidogenic pathway

APP cleavage by β-secretase (β-site APP cleaving enzyme/BACE1, also known as memapsin-2 and Asp2**)** near the membrane surface followed by γ-secretase cleavage within the cell membrane ultimately produces three peptide fragments. The initial cleavage of APP at the β-site yields two APP fragments. The N-terminal fragment is called soluble/secreted APP-β (sAPPβ), and the C-terminal fragment is called β-C-terminal fragment (β-CTF, also known as C99). Cleavage of the membrane-bound β-CTF by γ-secretase yields two more fragments: Aβ and γ-CTF (also known as APP intracellular domain, or AICD).

Depending on the actual site of γ-secretase cleavage, different isoforms of Aβ (39-43 amino acids long) will be produced. The shorter isoforms (e.g., $A\beta_{40}$) tend to exist in a soluble monomeric state. The hydrophobic C-terminals of the longer Aβ isoforms (e.g., $A\beta_{42}$) tend to cling together, forming oligomeric aggregates that eventually become deposits of insoluble Aβ plaque as they take on a β-sheet amyloid confirmation. These dense-core amyloid plaques are associated with neuritic dystrophy, suggesting that the process of polymerization from soluble, monomeric Aβ to insoluble amyloid appears to have a toxic effect on neurons. There is some evidence that sAPPβ and/or γ-CTF/AICD, the other APP peptide fragments produced by the amyloidogenic pathway, may have neuroprotective properties[39], and that γ-CTF can act as a

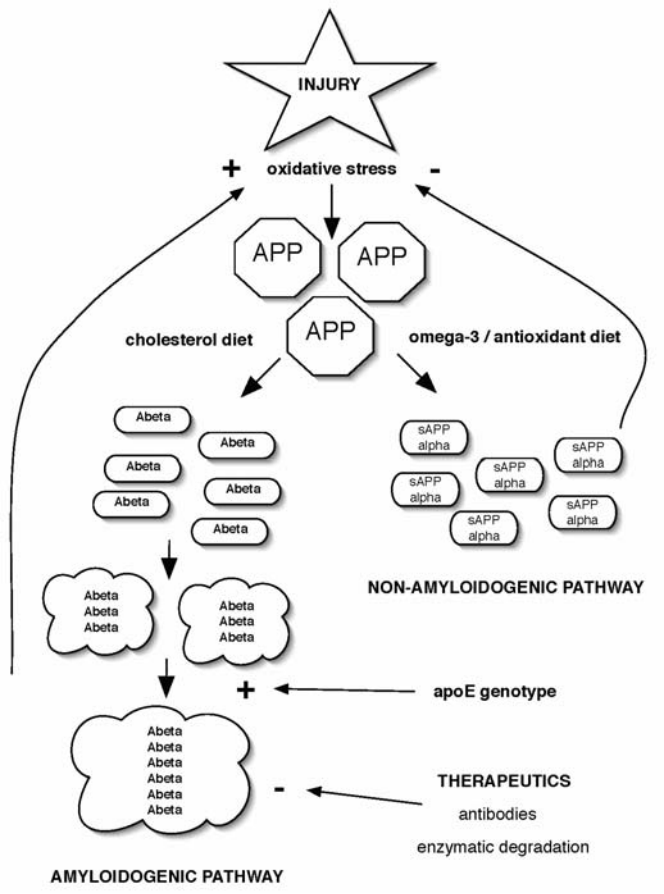
nuclear transcription factor[40,41]. There is also mounting evidence that the majority of APP processing actually occurs inside the cell[42] and that Aβ is released into the extracellular matrix via exocytosis during synaptic activity[43].

APP processing – Non-amyloidogenic pathway

A number of zinc metalloproteinases (sheddase / disintegrin / adamalysin proteins like ADAM9, ADAM10, ADAM17/TACE, BACE2) have been to shown to cleave APP at the α- site[32,44-50]. Because the α-secretase cleavage site lies within the Aβ domain of APP, α-secretase cleavage at the membrane surface [51] followed by γ-secretase cleavage within the membrane prevents production of Aβ and ultimately produces three peptide fragments. The first cleavage of APP by an α-secretase yields soluble/secreted APP-α (sAPPα) and α-CTF (also known as C83). Cleavage of the membrane-bound α-CTF by γ-secretase yields two more fragments: p3 (essentially a ~32 amino acid long subset of the Aβ protein) and γ-CTF/AICD.

Many studies have found that the α-secretase-cleaved APP ectodomain, sAPPα, has neuroprotective properties. For example, administration of exogenous sAPPα was neuroprotective against ischemia in rats[52]. This APP fragment is released from neurons in response to synaptic activity and may modulate neuronal excitability, synaptic plasticity, neurite growth, and cell survival. sAPPα has demonstrated effects on calcium homeostasis, potassium channels, N-methyl-D-aspartate (NMDA) glutamate receptors, and nuclear transcription factors[33,53,54]. Its neuroprotective mechanisms may involve raising the excitotoxicity threshold by stabilizing calcium levels[55-59]. Other putative mechanisms include the induction of gene expression for a number of neuroprotective proteins, including transthyretin (TTR), via interactions with nuclear factor-kappa B (NF-κB)[53]. In support of this idea, a transgenic mouse model of AD that expresses high levels of human APP and has high brain levels of Aβ, sAPPα and TTR (the Tg2576/APP_{sw} mouse) develops age-related dense-core amyloid plaques, but not NFTs or cell loss. When given an antibody against TTR, effectively removing or inactivating TTR in the brain, the APP_{sw} mice develop increased Aβ accumulation in the brain, intracellular tau phosphorylation, and neuronal apoptosis/death in the hippocampus, suggesting that sAPPα may exert part of its neuroprotective effects by inducing the upregulation of other neurotrophic and neuroprotective proteins[53].

Note that whereas both the β-secretase/amyloidogenic and the α-secretase non-amyloidogenic APP cleavage pathways produce fragments with potentially neuroprotective properties, the α-secretase pathway also prevents the formation of the potentially neurotoxic Aβ peptide. These neuroprotective fragments may provide an important clue as to the physiological role of APP and its cleavage products.



Accumulation of Aβ as a causative factor in AD

The gradual accumulation of Aβ in the brain appears to be associated with various downstream events causing functional neuronal deficits, structural brain damage, cognitive and behavioral impairments, and eventually death. Pathophysiological conditions that result in the accumulation of Aβ in the brain generally increase the risk of developing AD neuropathology. For example, Down syndrome, also known as trisomy 21, results from one extra copy of the 21st chromosome, which contains the *APP* gene. The condition is associated with the gene-dose dependent production of ~50% more APP than normal, leading to elevated Aβ production and deposition, and dementia by around 50 years of age[60,61].

Furthermore, over 30 genetic mutations have been identified in various families around the world that lead to early onset familial AD. These mutations are generally autosomal dominant and account for less than 5% of all AD cases. The mutations are either in the genes for APP or the presenilins (PS1 and PS2), which are membrane-bound constituents of γ-secretase. These familial AD mutations in the *APP* and *PSEN* genes all result in abnormally high levels of APP production and/or amyloidogenic APP processing. This, in turn, leads to the

increased production and deposition of Aβ (especially $A\beta_{42}$) in the brain and earlier onset of behavioral symptoms than in so-called "sporadic" AD[62,63].

Animals that model aspects of AD

The identification of genetic mutations associated with early-onset familial AD has spawned the development of several lines of transgenic mice that also express these mutations. These transgenic mouse models of AD generally have relatively high brain levels of mutant human APP and develop age-related cognitive deficits coincident with the formation of insoluble Aβ aggregates and Aβ amyloid deposits[64-71]. For example, one commonly used transgenic mouse model of AD is the Tg2576 (APP_{sw}) mouse. These transgenic mice express human APP from a Swedish family with autosomal dominant early-onset familial AD caused by a double mutation in the APP_{695} gene ($Lys^{670} \rightarrow Asn$ and $Met^{671} \rightarrow Leu$)[72]. Family members with these *APP* genetic mutations experience high levels of Aβ production and extensive accumulation of amyloid plaques (composed mostly of amyloidogenic $A\beta_{42}$[73]) in the brain. The age of disease onset in the family members with these mutations averages ~50 years.

APP_{sw} transgenic mice express the mutant human APP at roughly 5x the brain levels of wildtype mouse APP [74]. By approximately 6 months of age[68,74], these mice develop learning and memory deficits coincident with the formation of detergent-insoluble deposits of Aβ (especially $A\beta_{42}$)[75,76]. Amyloid plaques associated with dystrophic neurites start to form by 8 months of age [67-69]. Because soluble forms of Aβ in the brain are elevated by as early as 2 months in these mice, high brain levels of soluble Aβ are probably not solely responsible for the emergence of cognitive deficits at 6 months of age. In double-transgenic APP_{sw} mice that also produce human mutant PS1, both cognitive deficits and insoluble Aβ aggregates are detectable by as early as 2 months of age[76].

PDAPP transgenic mice express a different form of human mutant APP that is also associated with autosomal dominant familial AD resulting from the increased production of $A\beta_{42}$[77]. These transgenic mice also develop age-related learning deficits, accumulation of diffuse and neuritic plaques, glial activation, and abnormal phosphorylation of cytoskeletal microtubule-associated proteins beginning at around 6-9 months of age[78,79]. Thus, cognitive deficits associated with the appearance of insoluble Aβ aggregates have been reported in lines of transgenic mice in which the aggregates emerge at different ages.

Aβ accumulation causes AD neuropathology

Further evidence for the support of Aβ accumulation as a potential causative factor in AD comes from the observation that systemic treatments that lower brain levels of Aβ can prevent and reverse both behavioral and

neuronal dysfunction, as well as ameliorate intracellular tau pathology[80], in transgenic mouse models of AD[44,79,81-84]. For example, the systemic administration of monoclonal anti-Aβ antibodies to old PDAPP mice decreased Aβ deposition and improved both cognitive performance and neuronal function as measured by long-term potentiation (LTP)[79]. Additionally, dietary consumption of pomegranate juice, which contains very high levels of antioxidants and β-secretase inhibiting polyphenols, decreased Aβ deposition and improved cognitive performance in APP_{sw} mice[82]. These results strongly suggest that Aβ accumulation and the eventual appearance of some form of Aβ aggregate is responsible for the age-related neuronal dysfunction that eventually disrupts cognitive performance in these transgenic mice.

Thus, the ultimate cause of late-onset (sporadic) AD is currently unknown, but most likely has something to do with the abnormal buildup Aβ in the brain. The reason for Aβ accumulation in AD appears to be related to high levels of APP in the brain and/or excessive amyloidogenic APP processing. The accumulation of Aβ seems to induce a series of events that lead to even more Aβ accumulation, resulting in a vicious circle of neurodegenerative decline, known collectively as the amyloid cascade hypothesis of AD [85-87].

Risk factors

The most prevalent risk factor for the development of AD is aging. Other documented risk factors include genetics, diet, and brain injury.

Genetics as a risk factor

The autosomal dominant mutations in the genes that encode APP and the presenilins inevitably predispose individuals to develop early-onset familial AD by leading to either high levels of brain APP or excessive processing of APP via the amyloidogenic pathway. The major documented genetic risk factor for sporadic/late onset AD is carrying of a copy of the ε4 allele of the gene that encodes apolipoprotein E (apoE). ApoE is a low-density lipoprotein (LDL) that seems to play an important role in AD pathogenesis, and can also potentially interact with a number of different receptors as well as structures in the extracellular matrix.

Three major alleles of the *APOE* gene exist: ε2, ε3, and ε4. Carriers of the *APOE4* gene are more likely to develop AD, experiencing earlier and more pronounced Aβ deposition, hypercholesterolemia, and vascular dementia than individuals without the *APOE4* gene[88-92]. The increased AD risk is gene dose-dependent in that carriers of two copies of the *APOE4* gene have an even higher risk of developing AD than carriers of only one copy of *APOE4* [92-96]. Carrying a copy of the *APOE2* gene confers a degree of protection from Alzheimer's disease. The role of apoE has been investigated using *APOE* knockout ($APOE^{-/-}$)

mice, which express no apoE, and transgenic mice that express human apoE instead of mouse apoE. *APOE*$^{-/-}$ mice appear to be relatively normal, with no obvious cognitive impairment. Transgenic mice that express human apoE3 also seem to be unimpaired, whereas transgenic mice that express human apoE4 have profound learning and memory deficits in the absence of any obvious neuropathology[97,98], suggesting that apoE may play separate, but interacting, roles in the neuropathology and cognitive deficits observed in AD.

Mechanisms of APOE genotype as a risk factor

Although the mechanism of increased AD risk by an *APOE4* genotype is unclear, transgenic mice with human mutant APP have been crossed to transgenic mice that express different human apoE isoforms, allowing for the *in vivo* exploration of interactions between Aβ and apoE. ApoE is found co-localized with Aβ in plaques [99-104], and poorly lipidated apoE (produced in the absence of the lipid transporter ABCA1) is associated with the accelerated formation of insoluble Aβ and amyloid plaques in old PDAPP, *Abca*$^{-/-}$ mice [104].

Postmortem examination of synaptic density in the plaques of AD brains revealed that an *APOE4* genotype is associated with the fewest synapse-associated proteins, whereas the plaques of *APOE2* carriers have the highest synaptic density [25]. In both APP_{sw}, apoE$^{-/-}$ and PDAPP, apoE$^{-/-}$ mice (APP transgenic mice that also lack mouse apoE), Aβ deposition is significantly reduced, the vast majority of deposits are diffuse in nature (i.e., non-amyloid) and not associated with neuritic dystrophy [105-110], and cognitive deficits appear to be attenuated [111]. These findings suggest that apoE is somehow involved in the polymerization of soluble Aβ into a β-sheet amyloid structure, and that the reduction of cognitive deficits in these mice results from lower brain levels of aggregated Aβ (e.g., insoluble aggregates or soluble Aβ oligomers). Thus, the absence of apoE prevents aggregation of Aβ into toxic forms, and this may be linked to an attenuation of cognitive deficits in APP transgenic mice that lack apoE. Consequently, this evidence implies that the presence of apoE (especially apoE4) induces or enhances the aggregation of Aβ into toxic forms.

Besides playing a role in lipid transport, apoE and/or its peptide fragments appear to modulate the inflammatory response to brain injury, possibly by down-regulating the CNS anti-inflammatory and glial activation responses [112,113]. ApoE4, however, does not reduce inflammation efficiently as apoE3 [114], suggesting another mechanism by which an *APOE4* genotype can accentuate neuronal dysfunction. Furthermore, the introduction of iron into *in vitro* cultures of neuronal and vascular tissue produced oxidation of proteins and accumulation of Aβ and apoE in lysosomes and Aβ in the vasculature [115-118]. Tissue from an apoE4 lineage is significantly more affected than apoE3 tissue, suggesting that apoE4 may allow oxidative stress to go unchecked.

Diet as risk factor

Epidemiological and experimental evidence suggests that diet can alter the concentration of Aβ in the brain and consequently affect the risk for developing AD. For example, a high-cholesterol diet has been shown to elevate levels of both Aβ and apoE in the brains of rabbits (which express the human form of Aβ and naturally develop AD-like neuropathology with aging) [119] and APP transgenic mice [120]. Altering cholesterol and phospholipid levels in cell membranes can modulate the activity of membrane-bound enzymes, including the β- and γ-secretases that produce Aβ. These "amyloidogenic pathway" secretases seem to require cholesterol-rich "lipid rafts", or detergent-insoluble membrane domains (DIMs) within the membrane[121-124]. Conversely, α-secretase cleavage, which prevents the production of Aβ, requires a cholesterol-poor/phospholipid-rich, fluid membrane[47,125], thereby presenting one mechanism by which high cholesterol intake can lead to an increase in Aβ production and deposition. The LDLs (including apoE), which bind and transport cholesterol, are also highly sensitive to oxidative free radical damage, tending to aggregate, which impairs vascular perfusion and leads to hypoperfusion of the brain and increased risk of stroke. Similarly, high-carbohydrate diets can alter the metabolism of cellular membrane proteins (e.g., APP) similarly to the apoE4 protein [126], and conversely, diets high in omega-3 essential fatty acids and/or bioactive phytochemicals (e.g., polyphenols) have been shown to reduce AD-like neuropathology in transgenic mice [82,127-131].

Brain injury as a risk factor

Epidemiological and experimental evidence also suggests that a variety of types of brain injury may accelerate AD neuropathology and, consequently, the risk of developing AD. For example, traumatic brain injury (TBI) is associated with accelerated Aβ deposition and an increased risk of developing AD. Individuals who sustain moderate to severe head injury at some point in their life are more likely to develop AD and/or other forms of dementia [132-134], and Aβ deposition is found in the brains of approximately 1/3 of all people, regardless of age, who die shortly after TBI[135-138]. Professional athletes who experience repeated blows to the head, including boxers, football players, and soccer players, also have an increased risk of developing dementia (sometimes termed dementia pugilistica) with age[139-145]. Additionally, TBI patients are reported to have elevated levels of the amyloidogenic $A\beta_{42}$ in their cerebrospinal fluid for up to a week post-injury in comparison to both controls and AD patients[146-149]. These observations suggest that brain concentrations of Aβ spike transiently following traumatic brain injury, accelerating the aggregation process, eventually leading to worse neuropathology and earlier emergence of symptoms.

Other types of brain damage are also associated with AD-like neuropathology. Aβ plaque deposition in the cerebral arteries (cerebral amyloid angiopathy; CAA) can increase the risk of hemorrhagic or ischemic stroke[150-155]. Because of this, AD neuropathology has historically been seen as a risk factor for stroke. Indeed, the majority of autopsied brains with AD neuropathology also show evidence of stroke damage, leaving open the possibility that stroke damage can increase the risk for AD. Finally, Aβ deposition is also locally accelerated following seizure-induced neurodegeneration in human epileptic patients[156].

The effects of brain injury on AD neuropathology have also been studied using rats and APP transgenic mice[157]. It was first reported in 1991 that a stab injury to the brain elevated local levels of APP in rats[158]. Injury-induced local increases in Aβ concentration have been reported in a number of animal studies using both rodents and rabbits[159-162]. Relatively mild, but repetitive, cortical impact injury exacerbated levels of brain lipid peroxidation, Aβ deposition, and cognitive impairments in APP_{sw} mice[164,165]. Additionally, PDAPP transgenic mice lost 84% of their CA3 hippocampal neurons following experimental TBI, whereas wildtype control mice only lost 36%[163].

TBI induces brain inflammatory responses, oxidative stress, excitotoxic damage, apoptosis, astrogliosis, neuronal loss, and changes in brain vasculature. APP, microtubule-associated protein (MAP-2), and apoE accumulate in damaged white matter after TBI, indicating disrupted anterograde axonal transport and/or axotomy[166-169]. The post-injury accumulation of APP is consistent enough that APP immunohistochemistry is used as a marker for brain damage in experimental TBI models[170-172].

TBI also induces a transient increase in APP metabolism [173], which may prove to be neuroprotective if the APP is subsequently cleaved by α-secretase, leading to locally increased levels of the neuroprotective APP fragment sAPPα. When exogenous sAPPα was administered *in vivo* after TBI in rats, it improved motor function and reduced cellular apoptosis and axonal injury[174]. Interestingly, "preemptive" TBI induces neuroprotection to retinal ganglion cells from subsequent optic nerve crush-induced cell death, suggesting that brain damage may cause a systemic neuroprotective response to brain injury[175]. Oxidative stress following TBI may also play a role in elevated Aβ accumulation in the brain. Administration of an antioxidant (vitamin E) following TBI prevented the brain lipid peroxidation, elevated Aβ accumulation, and behavioral impairments observed in non-treated APP_{sw} mice[164].

Experimental stroke, including global cerebral ischemia and focal ischemia induced by middle cerebral artery occlusion, also induces the transient accumulation of APP and apoE in and around reactive astrocyte glial cells and damaged vasculature, axons, and neurons in the hippocampus, cortex,

cerebellum, basal ganglia and thalamus[99-102,176-178]. As with TBI, APP accumulation is also used as an immunohistochemical marker of experimental stroke-induced axonal damage. Short-term accumulation peaks within about 1 week of the injury, and tends to dissipate within a couple of weeks [179]. The accumulation appears to be due in part to APP upregulation at the mRNA level [180-185] in addition to other factors[186]. Similar to TBI, stroke also causes a high degree of oxidative stress, suggesting another mechanism by which APP and Aβ may be modulated. Transient hypoxia in cultured cortical neurons induces a number of biochemical processes that are common to AD[187], including mitochondrial dysfunction, neuronal membrane damage, apoptosis, and APP cleavage.

As noted, human epileptic seizures are also associated with Aβ accumulation in the epileptogenic tissue[156]. During a seizure or other brain insult, the excitatory neurotransmitter glutamate accumulates in the extracellular space, causing over-stimulation of neurons and leading to apoptotic cell death. This process, termed excitotoxicity [188], is often studied experimentally using kainic acid. Kainic acid-induced excitotoxic damage causes increased expression of glial-produced APP, but a decrease in neuronal-produced APP in rats. These effects were prevented by MK-801 and pentobarbitone, drugs that prevent excitotoxic damage[189]. The release of intracellular Aβ during injury-induced heightened synaptic activity[43] provides yet another mechanism by which a host of brain injuries may lead to the ultimate accumulation of Aβ, causing an increased risk of AD.

Risk factor interactions

Epidemiological and experimental evidence suggests that carrying at least one copy of the *APOE4* allele can interact synergistically with the effects of brain injury, increasing both the acute and chronic consequences of the injury in addition to increasing the risk of developing AD. Human studies have shown that both short- and long-term behavioral recovery from TBI can be influenced by *APOE* genotype. For example, *APOE4* carriers scored significantly worse than *APOE4*- individuals on neuropsychological tests 3 weeks after mild to moderate TBI[190], and *APOE4*+ TBI patients experienced longer periods of unconsciousness and worse clinical outcome[191]. *APOE4* carriers were twice as likely as *APOE4*- individuals to be either dead, comatose, or severely disabled 6 months following TBI[192]. In addition to a generally worse clinical outcome, the long-term effects of TBI on memory[193] and motor[194] performance are significantly worse for *APOE4* carriers. Mild, but repetitive, head injury also appears to interact with apoE. *APOE4*+ professional boxers had significantly worse cognitive and motor scores on a neurological test of chronic brain injury than *APOE4*- boxers[195]. Similarly, older *APOE4*+ professional football players scored lower on cognitive tests than *APOE4*- players[196].

Additionally, the risk of developing AD following brain injury is dramatically heightened by an *APOE4*+ genotype, in that *APOE4*+ individuals are even more likely to develop dementia if they sustain TBI sometime in their life. The risk for developing AD associated with an *APOE4*+ genotype quintuples from 2x to 10x following a TBI[132,197]. TBI is associated with accelerated Aβ deposition in the brain's parenchyma and vasculature[198], with an even greater effect observed in *APOE4*+ individuals[199-201]. Additionally, a significant proportion of the people with Aβ deposition in their brain shortly after dying from TBI have at least one copy of the *APOE4* allele[138,198,200,201]. Aβ deposition can also be found in the brains of even young *APOE4*+ epilepsy patients, who experience excitotoxic neurodegeneration and locally increased synaptic activity as a result of their seizures[156].

Subjecting transgenic mice that express human APP and/or apoE isoforms to various models of brain injury has elucidated the interactions between these major risk factors for AD. In an experiment evaluating the effects of TBI on *APOE3* versus *APOE4* transgenic mice, greater mortality and worse behavioral outcomes, but no differences in the amount of tissue loss, were observed in the *APOE4* mice [202]. Additionally, within 24 hours of a closed head injury insult, *APOE4* transgenic mice expressed less sAPPα than *APOE3* mice[173].

Studies of APP x apoE transgenic mice have provided additional evidence that brain injury can interact with Aβ and an *APOE4* genotype to accelerate AD neuropathology. PDAPP transgenic mice that also express human *APOE3* or *APOE4* were subjected to a cortical impact TBI and later assessed for TBI-induced neurodegeneration and AD-like neuropathology[203]. Although no differences were found in the amount of cortical or hippocampal cell loss, Aβ deposition following TBI was accelerated in PDAPP, *APOE4* mice as compared to PDAPP, *APOE3* mice or PDAPP, *APOE*-/- mice. The finding that TBI accelerated the Aβ deposition process in the presence of human apoE4 as compared to apoE3 suggests that TBI and apoE4 interact to result in not only higher local concentrations of Aβ, but also earlier aggregation.

Overall, these data suggest that the association between *APOE4* and higher risk for functional outcome and/or AD following TBI may in part be due to isoform-specific APOE/Aβ interactions contributing to the premature development of AD neuropathology.

Mechanisms of the apoE X brain injury interaction

It is not yet clear how brain injury can result in an apoE isoform-dependent increase in amyloid deposition. Some *in vivo* studies have found that apoE can influence aspects of brain function and plasticity following different types of brain injury[202,204-209], and it is probable that apoE influences outcome after brain injury via more than one mechanism.

A number of both *in vitro* and *in vivo* studies have demonstrated that apoE interacts with Aβ and influences the probability of Aβ aggregation into a β-sheet/amyloid/neurotoxic conformation[210,211]. The levels of apoE in the brain may play a significant role in this effect, as the presence of mouse apoE increases Aβ deposition in a gene dose dependent fashion *in vivo*[212]. Additionally, brain levels of apoE are elevated, coincident with glial activation, after a variety of brain injury paradigms[213]. Thus, the effects of TBI on apoE/Aβ interactions may be the result of local injury-induced increases in the brain concentrations of apoE, APP, and Aβ.

General mechanisms of elevated Aβ/APP

Mounting evidence suggests that brain inflammation and oxidative stress resulting from TBI, stroke, or even chronic low-level insult (e.g., hypoxia due to breathing problems[214]) induces the accumulation of APP in the brain. Possible mechanisms of elevated APP, Aβ, and apoE levels following brain injury include upregulation, release of intracellular peptides, passage from the periphery through an injury-compromised blood-brain barrier[100,215-225], and/or injury-induced problems with clearance. Oxidative stress, a common component of all types of brain injury, is sufficient to induce Aβ accumulation[115], and some evidence suggests that insult-induced microglial activation ultimately causes upregulation and accumulation of APP[87,226-229].

Whatever the mechanism, if the non-amyloidogenic α-secretase pathway metabolizes the excess APP, then the subsequent production of sAPPα fragments could have a significant neuroprotective effect by a number of mechanisms. If the local environment promotes the amyloidogenic β-secretase cleavage pathway (e.g., high cholesterol/low phospholipid membrane content), the potential beneficial impact of elevated APP levels could be reduced or reversed, and may increase the risk of developing AD neuropathology via accumulation of Aβ. Thus, APP accumulation in response to acute brain damage, as well as chronic inflammation, may play an important role in the development of AD. This would suggest several possible preventative/therapeutic treatment strategies, including prophylaxis, attenuating the accumulation of Aβ in the brain, and neutralizing the neurotoxic effects of aggregated Aβ via degradation and/or clearance from the brain.

Therapeutic implications

1. Reduce risk of insult-induced brain damage by maintaining a high antioxidant, low cholesterol diet

Diet has the potential to effect sporadic Aβ accumulation over the lifespan and to reduce risk of long-term damage as a result of an insult to the brain by a

number of mechanisms. For example, caloric restriction appears to be neuroprotective in APP transgenic mice[230,231], perhaps by decreasing the accumulation of Aβ deposits [232]. Mounting epidemiological evidence suggests that diet can decrease the risk for developing AD[233-235].

A diet high in antioxidants has the potential to attenuate both basal and injury-induced oxidative stress. Given that Aβ accumulation seems to cause oxidative stress and inflammation, leading to the accumulation of even more Aβ, keeping free radical-induced oxidative stress in check may reduce the slow, but steady, accumulation of Aβ in the brain with aging[236,237]. Epidemiological evidence suggests that high intake of food-based vitamin E is associated with a lower incidence of AD in humans[238], and chronic dietary administration of the antioxidant vitamin E to young (but not old) APP_{sw} mice reduced Aβ deposition[239]. Dietary administration of the antioxidant vitamin E to APP_{sw} mice before and after repetitive TBI ameliorated oxidative stress, injury-accelerated Aβ formation, and behavioral impairments, suggesting that oxidative stress induced by brain injury plays a mechanistic role in the risk for AD and that this risk can be reduced with a high antioxidant diet[164].

Phytochemicals like polyphenols (including the phenolic acids and flavonoids) are bioactive chemicals found in plants (especially pigments). Polyphenols have antioxidant and anti-inflammatory properties, as well as effects on nitric oxide synthase production and other signaling pathways[240,241]. Isolated dietary polyphenols have reduced Aβ deposition and/or improved cognitive performance in APP transgenic mice. For example, curcumin, a polyphenol found in the curry spice turmeric, was shown to lower levels of oxidized proteins and plaque burden in APP_{sw} mice[242]. Epigallocatechin-3-gallate (EGCG), a polyphenolic component of green tea, reduced production of Aβ and elevated levels of α-secretase processing *in vitro*, and decreased levels of Aβ and plaque deposition in the brains of APP_{sw} mice[243]. Tannic acids, also found in tea, have been shown to inhibit amyloid fibril formation *in vitro*[244]. Resveratrol, a polyphenol found in grapes and red wine, was shown to decrease levels of Aβ *in vitro* by increasing clearance, rather than inhibiting production, of Aβ[245]. Aβ-heme complexes found within plaques can cause oxidative stress and damage to muscarinic ACh receptors, which promote α-secretase activity. This damage can be ameliorated by polyphenols and other antioxidants[10,11,16].

Importantly, whole foods often contain a variety of phytochemicals that may work together synergistically [246,247]. Epidemiological evidence shows that consumption of green tea, a food high in polyphenols, may be neuroprotective [248], and dietary consumption of fruits and vegetables may decrease risk of AD [249-252].

Animal studies have shown that dietary supplementation with foods high in polyphenols can also affect both neuropathology and behavior.

Supplementation of chow with polyphenol-rich blueberries did not decrease Aβ plaque levels, but improved cognitive performance, in double transgenic APP, PS1 mice[253]. Dietary supplementation of pregnant mice with pomegranate juice, another food with very high concentrations of polyphenols[254,255], protected against neurodegeneration in the neonatal offspring when subjected to hypoxic-ischemic brain injury[256]. Pomegranate juice also reduced levels of soluble $A\beta_{42}$, Aβ deposition, and fibrillar Aβ/amyloid deposition in the hippocampi of APP_{sw} mice[82]. In addition to the antioxidant effects of polyphenolic phytochemicals, ellagic acid extracted from pomegranate husks can inhibit β-secretase activity *in vitro*[257]. Thus, it appears that antioxidants and various naturally occurring dietary phytochemicals can decrease the levels of soluble and deposited Aβ in the brain, possibly by inhibiting production, disrupting aggregation, or enhancing clearance of Aβ.

Additionally, phospholipids like the omega-3 fatty acids increase membrane fluidity, promoting α-secretase processing of APP and the formation of neuroprotective sAPPα, whereas cholesterol can decrease membrane fluidity and promote β-secretase processing of APP and the formation of neurotoxic Aβ. Diets high in omega-3 fatty acids and low in cholesterol should therefore promote α-secretase (and inhibit β-secretase) processing of both basal and insult-induced APP[125], leading to the generation of less harmful Aβ and more neuroprotective sAPPα[127,129]. For individuals with very high cholesterol levels, reduction via drugs like the statins may also provide some benefit [258].

The omega-3 essential fatty acids such as those contained in fish oil (e.g., docosahexaenoic acid / DHA) may be neuroprotective in humans[259-263]. DHA comprises around 15% of the brain's total fatty acids, and 30-40% of the gray matter. Rodent studies have also shown beneficial effects of dietary DHA on learning in a rat model of AD[264,265] and on both plaque deposition and dendritic pathology in aged APP_{sw} mice[127,129].

Thus, a diet high in antioxidants and omega-3 fatty acids and low in cholesterol should maintain a brain environment that is relatively resistant to the effects of brain damage following insult. The prophylactic mechanisms may work through a number of pathways, including attenuation of oxidative stress and inflammation, promotion of α-secretase APP processing/production of neuroprotective sAPPα, and inhibition of β-secretase APP processing/production of neurotoxic Aβ. Other prophylactic strategies may involve regular exercise and the use of non-steroidal anti-inflammatory drugs[84,266-269].

2. Prevent Aβ accumulation following injury

In the event of an acute brain injury, efforts should next be made to deal with the newly increased risk of Aβ accumulation. Because of the evidence

that α-secretase processing of APP both produces neuroprotective sAPPα and prevents the formation of potentially toxic Aβ, promotion of α-secretase processing may prove to be an effective strategy for dealing with the locally elevated concentrations of APP that tend to follow brain injury. *In vivo* application of exogenous sAPPα had positive effects on functional motor outcome, cellular apoptosis, and axonal injury following TBI in rats[174]. Therapeutic strategies that promote α-secretase processing could have effects on Aβ accumulation via multiple pathways[45,47]. A number of compounds are associated with an upregulation of α-secretase processing[46], including protein kinase C activators (like phorbol esters)[270], various growth factors**,** cholesterol-lowering drugs, steroid hormones, non-steroidal anti-inflammatory drugs[268,271], metal ions, and phytochemicals[272]. ACh muscarinic agonists also promote α-secretase via interactions with M1 and M3 receptors[273-276]. These receptors are damaged by oxidative stress from Aβ-heme complexes, and this damage is ameliorated by dietary polyphenols[16], providing yet another mechanism by which diet could effect the risk for AD before and following brain damage.

In addition to stimulation of the non-amyloidogenic α-secretase APP cleavage pathway, β- and/or γ-secretase inhibition represents another potential strategy for dealing with the accumulation of APP following brain injury[277-279]. For example, γ-secretase inhibitors can decrease plaque load in APP_{sw} transgenic mice[280] and provide neuroprotection to cultured cortical neurons from hypoxia-induced increases in mitochondrial dysfunction, neuronal membrane damage, apoptosis, and APP cleavage[187]. It is worthy to note, however, that γ-CTF/AICD, one of the byproducts of γ-secretase processing, acts as a nuclear transcription factor and affects calcium signaling[281,282]. It is unclear how inhibiting the production of this APP fragment could change other cellular parameters such as excitotoxic threshold.

3. Clear and/or neutralize Aβ after injury

Once β-secretase and γ-secretase cleave APP and produce Aβ in high enough concentrations, it appears inevitable that the longer isoforms (e.g., $Aβ_{42}$) will begin to clump together, forming oligomeric aggregates and eventually dense-core amyloid plaques. This implies that after a brain injury, Aβ clearance, degradation, and/or aggregation inhibition should be the next therapeutic target. Some evidence suggests that soluble and insoluble Aβ is constantly degraded by a number of proteases in the extracellular matrix and interstitial fluid. Zinc metalloproteinases, including endothelin-converting enzymes, insulin-degrading enzyme, and neprilysin have all been shown to degrade soluble Aβ, and metalloproteinase-9 can degrade aggregated fibrillar Aβ[283,284]. This suggests a degree of dynamic equilibrium in the brain for Aβ

and amyloid plaques, and provides another target for therapeutic interactions. These strategies should be carefully evaluated, as it is possible that dissolving deposited fibrillar Aβ could reintroduce potentially toxic oligomeric Aβ aggregates to the surrounding brain tissue.

Several studies have assessed the effects of immunotherapeutic treatments targeting Aβ[266,285]. For instance, active immunization with Aβ and Aβ fragments can reduce Aβ deposition, astrogliosis, and learning deficits in APP transgenic mice[286-290]. In a study of active immunization of human AD patients with Aβ, the treatment appeared to have had a small effect on Aβ levels, but also seemed to have induced fatal encephalitis, micro-hemorrhages, and other adverse effects[291]. Passive immunization with monoclonal anti-AB antibodies eliminates the potentially harmful auto-immunological responses of active immunization and may thus represent a safer therapeutic strategy[292,293]. Several studies have now confirmed that passive immunization of APP transgenic mice with Aβ-targeting monoclonal antibodies can reduce brain concentrations of several forms of Aβ, leading to improved neuronal function and cognition.

Old PDAPP mice given the monoclonal Aβ antibody m266 (which binds to the central domain of soluble, non-fibrillar Aβ) exhibited improved cognition, but no observable effect on Aβ deposition, within 24-72 hours[81]. Additionally, middle- and old-aged PPAPP mice treated for weeks to months with the monoclonal N-terminal Aβ antibody 10D5 accumulated 50% less diffuse and fibrillar Aβ in the hippocampus and exhibited improved spatial learning. Furthermore, hippocampal LTP, a measure of synaptic efficacy, was normal in 10D5-treated PDAPP mice, but deficient in untreated PDAPP mice[79].

Nine to 11-month old APP_{sw} mice treated with the monoclonal N-terminal Aβ antibody BAM10 for several days demonstrated improved cognition but no reduction in Aβ deposition levels[83], and another study[294] reported improved exploratory performance and reduced Aβ deposition in old (~22-month) APP_{sw} mice after 3 months of treatment with the monoclonal Aβ antibody 2286. Both active and passive immunization was also shown to prevent synaptic loss in APP_{sw} mice[295]. NAB61, an antibody specific to oligomeric Aβ, improved cognition but did not reduce plaque load[296].

These effects of passive monoclonal Aβ antibody treatment provide direct evidence for the detrimental cognitive effects of Aβ aggregation and deposition. The behavioral effects in transgenic mice seem to be evident by the first several days of testing, but the effects on Aβ deposition and/or clearance seem to take at least several weeks. This suggests that the clearance of Aβ soluble aggregates may have a direct and immediate effect on neuronal function.

Although the various anti-Aβ antibodies all bind to Aβ, they bind at different epitopes and to different conformations, and the mechanism of Aβ clearance after peripheral monoclonal antibody treatment remains to be

determined. Possibilities include the antibody binding to: 1) Aβ plaques in the brain, leading to microglial activation and phagocytosis[297,294, 298], 2) Aβ plaques in the brain, leading to plaque disruption[297,299], 3) soluble, non-deposited Aβ in the brain, disrupting aggregation[81], and/or 4) soluble Aβ in the peripheral, causing a peripheral "soluble Aβ sink"[79,293,300,301]. Given that evidence exists for all of these potential mechanisms, it is possible that a cocktail of various monoclonal antibodies may provide the most effective results. Regardless of the mechanism, studies on the effects of passive immunization with monoclonal Aβ antibodies support the idea that targeting Aβ with antibodies may be a viable treatment for AD, and that this treatment strategy has a chance to be effective and safe. Caution should be exercised, however, as certain N-terminal Aβ antibodies that can bind to fibrillar Aβ have the potential to weaken amyloid-ridden blood vessels, leading to micro-hemorrhage[301,302]. Another interesting approach involves gene therapy, in which adeno-associated virus vectors cause the expression of Aβ antibody fragments that can bind to Aβ, but lack the ability to activate a microglial immune response[303,304].

Summary

The accumulation of Aβ in the brain is a causative factor for Alzheimer's disease, and evidence suggests that a variety of brain injuries may increase the risk for AD. People with traumatic brain injury, seizures or stroke are more likely to develop Aβ plaques in their brains. Even children may quickly develop AD-like plaques following TBI. The most common cause of brain injury in the elderly is stroke, and most AD brains exhibit stroke pathology at autopsy. Animal experiments suggest that brain damage elevates levels of APP, and consequently Aβ, and accelerates plaque deposition. It is possible that brain damage initiates or accelerates AD pathology by upregulated APP and ApoE (a protein involved in transforming soluble Aβ into plaques), decreased clearance of Aβ, blood-brain barrier dysfunction (allowing peripheral Aβ to enter the brain), and/or increased neuronal release of Aβ during damage-induced synaptic activity. Thus, brain damage may trigger a cascade of physiological events leading to AD. Because AD pathology includes buildup of vascular Aβ, and because this increases the risk of future stroke, the process may self-propagate. If early efforts are made to prevent excessive Aβ deposition after acute brain damage such as TBI or stroke, it may be possible to delay the onset of AD. Because AD is a progressive disease of the elderly, delaying the onset by only a few years would significantly decrease its prevalence.

Currently, a multifaceted therapeutic approach appears to have the best chance of reducing the risk of AD following brain injury. A consistently high antioxidant, low cholesterol diet may help to attenuate brain damage following

insult. After a damaging event, efforts should be made to reduce the overproduction of Aβ, perhaps by promoting α-secretase cleavage, thereby inducing sAPPα and inhibiting Aβ production, or by inhibiting γ- or β-secretase processing. Residual Aβ may be dealt with using anti-Aβ monoclonal antibodies or promoting Aβ proteolytic degradation and/or clearance. Because stroke is a relatively common disorder of aging, preventing the increased accumulation of AD neuropathology is of utmost importance in light of the fact that low-level background Aβ deposition seems to occur over the lifetime of the individual. Additionally, because TBI often occurs in young patients, and AD is a disease of aging, there may be ample time to prevent or reverse the AD-like neuropathology associated with brain injury early in life.

References

1. Goldman, W. P. & Morris, J. C. Evidence that age-associated memory impairment is not a normal variant of aging. *Alzheimer Dis Assoc Disord* **15**, 72-79 (2001).
2. Goldman, W. P. et al. Absence of cognitive impairment or decline in preclinical Alzheimer's disease. *Neurology* **56**, 361-367 (2001).
3. Morris, J. C. & Price, A. L. Pathologic correlates of nondemented aging, mild cognitive impairment, and early-stage Alzheimer's disease. *J Mol Neurosci* **17**, 101-118 (2001).
4. Albert, M. S. Parallels between Down syndrome dementia and Alzheimer's disease. *Prog Clin Biol Res* **379**, 77-102 (1992).
5. Scott, B. S., Becker, L. E. & Petit, T. L. Neurobiology of Down's syndrome. *Prog Neurobiol* **21**, 199-237 (1983).
6. Alexander, G. E. et al. Association of premorbid intellectual function with cerebral metabolism in Alzheimer's disease: implications for the cognitive reserve hypothesis. *Am J Psychiatry* **154**, 165-172 (1997).
7. Roe, C. M., Xiong, C., Miller, J. P. & Morris, J. C. Education and Alzheimer disease without dementia: support for the cognitive reserve hypothesis. *Neurology* **68**, 223-228 (2007).
8. Stern, Y. Cognitive reserve and Alzheimer disease. *Alzheimer Dis Assoc Disord* **20**, 112-117 (2006).
9. Glenner, G. G. & Wong, C. W. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem Biophys Res Commun* **120**, 885-890 (1984).
10. Atamna, H. & Boyle, K. Amyloid-beta peptide binds with heme to form a peroxidase: relationship to the cytopathologies of Alzheimer's disease. *Proc Natl Acad Sci U S A* **103**, 3381-3386 (2006).
11. Atamna, H. & Frey, W. H. n. A role for heme in Alzheimer's disease: heme binds amyloid beta and has altered metabolism. *Proc Natl Acad Sci U S A* **101**, 11153-11158 (2004).
12. Mann, D. M. The pathogenesis and progression of the pathological changes of Alzheimer's disease. *Ann Med* **21**, 133-136 (1989).

13. Lanari, A., Amenta, F., Silvestrelli, G., Tomassoni, D. & Parnetti, L. Neurotransmitter deficits in behavioural and psychological symptoms of Alzheimer's disease. *Mech Ageing Dev* **127**, 158-165 (2006).
14. Reddy, P. H. Amyloid precursor protein-mediated free radicals and oxidative damage: implications for the development and progression of Alzheimer's disease. *J Neurochem* **96**, 1-13 (2006).
15. Reddy, P. H. et al. Gene expression profiles of transcripts in amyloid precursor protein transgenic mice: up-regulation of mitochondrial metabolism and apoptotic genes is an early cellular change in Alzheimer's disease. *Hum Mol Genet* **13**, 1225-1240 (2004).
16. Fawcett, J. R. et al. Inactivation of the human brain muscarinic acetylcholine receptor by oxidative damage catalyzed by a low molecular weight endogenous inhibitor from Alzheimer's brain is prevented by pyrophosphate analogs, bioflavonoids and other antioxidants. *Brain Res* **950**, 10-20 (2002).
17. Stein, T. D. et al. Neutralization of transthyretin reverses the neuroprotective effects of secreted amyloid precursor protein (APP) in APPSW mice resulting in tau phosphorylation and loss of hippocampal neurons: support for the amyloid hypothesis. *J Neurosci* **24**, 7707-7717 (2004).
18. Wenk, G. L. Neuropathologic changes in Alzheimer's disease: potential targets for treatment. *J Clin Psychiatry* **67 Suppl 3**, 3-7; quiz 23 (2006).
19. Rowan, M. J., Klyubin, I., Cullen, W. K. & Anwyl, R. Synaptic plasticity in animal models of early Alzheimer's disease. *Philos Trans R Soc Lond B Biol Sci* **358**, 821-828 (2003).
20. Rowan, M. J., Klyubin, I., Wang, Q. & Anwyl, R. Mechanisms of the inhibitory effects of amyloid beta-protein on synaptic plasticity. *Exp Gerontol* **39**, 1661-1667 (2004).
21. Rowan, M. J., Klyubin, I., Wang, Q. & Anwyl, R. Synaptic plasticity disruption by amyloid beta protein: modulation by potential Alzheimer's disease modifying therapies. *Biochem Soc Trans* **33**, 563-567 (2005).
22. Bisel, B. E., Henkins, K. M. & Parfitt, K. D. Alzheimer amyloid beta-peptide A-beta25-35 blocks adenylate cyclase-mediated forms of hippocampal long-term potentiation. *Ann N Y Acad Sci* **1097**, 58-63 (2007).
23. Dong, H., Martin, M. V., Chambers, S. & Csernansky, J. G. Spatial relationship between synapse loss and beta-amyloid deposition in Tg2576 mice. *J Comp Neurol* **500**, 311-321 (2007).
24. Lacor, P. N. et al. Abeta oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer's disease. *J Neurosci* **27**, 796-807 (2007).
25. Love, S. et al. Premorbid effects of APOE on synaptic proteins in human temporal neocortex. *Neurobiol Aging* **27**, 797-803 (2006).
26. Scheff, S. W., Price, D. A., Schmitt, F. A., DeKosky, S. T. & Mufson, E. J. Synaptic alterations in CA1 in mild Alzheimer disease and mild cognitive impairment. *Neurology* **68**, 1501-1508 (2007).
27. Brendza, R. P. et al. PDAPP; YFP double transgenic mice: a tool to study amyloid-beta associated changes in axonal, dendritic, and synaptic structures. *J Comp Neurol* **456**, 375-383 (2003).

28. Grutzendler, J., Helmin, K., Tsai, J. & Gan, W. B. Various dendritic abnormalities are associated with fibrillar amyloid deposits in Alzheimer's disease. *Ann N Y Acad Sci* **1097**, 30-39 (2007).
29. Cheng, I. H. et al. Accelerating amyloid-beta fibrillization reduces oligomer levels and functional deficits in Alzheimer disease mouse models. *J Biol Chem* (2007).
30. Walsh, D. M. et al. Amyloid beta-protein fibrillogenesis. Structure and biological activity of protofibrillar intermediates. *J Biol Chem* **274**, 25945-25952 (1999).
31. Walsh, D. M., Lomakin, A., Benedek, G. B., Condron, M. M. & Teplow, D. B. Amyloid beta-protein fibrillogenesis. Detection of a protofibrillar intermediate. *J Biol Chem* **272**, 22364-22372 (1997).
32. Turner, P. R., O'Connor, K., Tate, W. P. & Abraham, W. C. Roles of amyloid precursor protein and its fragments in regulating neural activity, plasticity and memory. *Prog Neurobiol* **70**, 1-32 (2003).
33. Mattson, M. P. Cellular actions of beta-amyloid precursor protein and its soluble and fibrillogenic derivatives. *Physiol Rev* **77**, 1081-1132 (1997).
34. Steinbach, J. P. et al. Hypersensitivity to seizures in beta-amyloid precursor protein deficient mice. *Cell Death Differ* **5**, 858-866 (1998).
35. Phinney, A. L. et al. No hippocampal neuron or synaptic bouton loss in learning-impaired aged beta-amyloid precursor protein-null mice. *Neuroscience* **90**, 1207-1216 (1999).
36. von Koch, C. S. et al. Generation of APLP2 KO mice and early postnatal lethality in APLP2/APP double KO mice. *Neurobiol Aging* **18**, 661-669 (1997).
37. De Strooper, B. & Annaert, W. Proteolytic processing and cell biological functions of the amyloid precursor protein. *J Cell Sci* **113**, 1857-1870 (2000).
38. Sisodia, S. S., Koo, E. H., Beyreuther, K., Unterbeck, A. & Price, D. L. Evidence that beta-amyloid protein in Alzheimer's disease is not derived by normal processing. *Science* **248**, 492-495 (1990).
39. Ma, H. et al. Involvement of beta-site APP cleaving enzyme 1 (BACE1) in amyloid precursor protein-mediated enhancement of memory and activity-dependent synaptic plasticity. *Proc Natl Acad Sci U S A* **104**, 8167-8172 (2007).
40. Cao, X. & Sudhof, T. C. A transcriptionally [correction of transcriptively] active complex of APP with Fe65 and histone acetyltransferase Tip60. *Science* **293**, 115-120 (2001).
41. Cao, X. & Sudhof, T. C. Dissection of amyloid-beta precursor protein-dependent transcriptional transactivation. *J Biol Chem* **279**, 24601-24611 (2004).
42. Vetrivel, K. S. & Thinakaran, G. Amyloidogenic processing of beta-amyloid precursor protein in intracellular compartments. *Neurology* **66**, S69-73 (2006).
43. Cirrito, J. R. et al. Synaptic activity regulates interstitial fluid amyloid-beta levels in vivo. *Neuron* **48**, 913-922 (2005).
44. Allinson, T. M. et al. The role of ADAM10 and ADAM17 in the ectodomain shedding of angiotensin converting enzyme and the amyloid precursor protein. *Eur J Biochem* **271**, 2539-2547 (2004).
45. Allinson, T. M., Parkin, E. T., Turner, A. J. & Hooper, N. M. ADAMs family members as amyloid precursor protein alpha-secretases. *J Neurosci Res* **74**, 342-352 (2003).
46. Hooper, N. M. & Turner, A. J. The search for alpha-secretase and its potential as a therapeutic approach to Alzheimer s disease. *Curr Med Chem* **9**, 1107-1119 (2002).

47. Kojro, E. & Fahrenholz, F. The non-amyloidogenic pathway: structure and function of alpha-secretases. *Subcell Biochem* **38**, 105-127 (2005).
48. Kojro, E. et al. The neuropeptide PACAP promotes the alpha-secretase pathway for processing the Alzheimer amyloid precursor protein. *FASEB J* **20**, 512-514 (2006).
49. Lammich, S. et al. Constitutive and regulated alpha-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. *Proc Natl Acad Sci U S A* **96**, 3922-3927 (1999).
50. Postina, R. et al. A disintegrin-metalloproteinase prevents amyloid plaque formation and hippocampal defects in an Alzheimer disease mouse model. *J Clin Invest* **113**, 1456-1464 (2004).
51. Parvathy, S., Hussain, I., Karran, E. H., Turner, A. J. & Hooper, N. M. Cleavage of Alzheimer's amyloid precursor protein by alpha-secretase occurs at the surface of neuronal cells. *Biochemistry* **38**, 9728-9734 (1999).
52. Smith-Swintosky, V. L. et al. Secreted forms of beta-amyloid precursor protein protect against ischemic brain injury. *J Neurochem* **63**, 781-784 (1994).
53. Guo, Q., Robinson, N. & Mattson, M. P. Secreted beta-amyloid precursor protein counteracts the proapoptotic action of mutant presenilin-1 by activation of NF-kappaB and stabilization of calcium homeostasis. *J Biol Chem* **273**, 12341-12351 (1998).
54. Mattson, M. P. et al. Cellular signaling roles of TGF beta, TNF alpha and beta APP in brain injury responses and Alzheimer's disease. *Brain Res Brain Res Rev* **23**, 47-61 (1997).
55. Barger, S. W., Smith-Swintosky, V. L., Rydel, R. E. & Mattson, M. P. beta-Amyloid precursor protein mismetabolism and loss of calcium homeostasis in Alzheimer's disease. *Ann N Y Acad Sci* **695**, 158-164 (1993).
56. Mattson, M. P. et al. beta-Amyloid precursor protein metabolites and loss of neuronal Ca2+ homeostasis in Alzheimer's disease. *Trends Neurosci* **16**, 409-414 (1993).
57. Mattson, M. P. et al. Evidence for excitoprotective and intraneuronal calcium-regulating roles for secreted forms of the beta-amyloid precursor protein. *Neuron* **10**, 243-254 (1993).
58. Mattson, M. P. & Rydel, R. E. beta-Amyloid precursor protein and Alzheimer's disease: the peptide plot thickens. *Neurobiol Aging* **13**, 617-621 (1992).
59. Mattson, M. P., Rydel, R. E., Lieberburg, I. & Smith-Swintosky, V. L. Altered calcium signaling and neuronal injury: stroke and Alzheimer's disease as examples. *Ann N Y Acad Sci* **679**, 1-21 (1993).
60. Head, E. & Lott, I. T. Down syndrome and beta-amyloid deposition. *Curr Opin Neurol* **17**, 95-100 (2004).
61. Mehta, P. D., Capone, G., Jewell, A. & Freedland, R. L. Increased amyloid beta protein levels in children and adolescents with Down syndrome. *J Neurol Sci* **254**, 22-27 (2007).
62. Selkoe, D. J. The origins of Alzheimer disease: a is for amyloid. *JAMA* **283**, 1615-1617 (2000).
63. Selkoe, D. J. Alzheimer's disease results from the cerebral accumulation and cytotoxicity of amyloid beta-protein. *J Alzheimers Dis* **3**, 75-80 (2001).
64. Arendash, G. W. et al. Progressive, age-related behavioral impairments in transgenic mice carrying both mutant amyloid precursor protein and presenilin-1 transgenes. *Brain Res* **891**, 42-53 (2001).

65. Dodart, J. C., Mathis, C., Bales, K. R. & Paul, S. M. Does my mouse have Alzheimer's disease? *Genes Brain Behav* **1**, 142-155 (2002).
66. Gordon, M. N. et al. Correlation between cognitive deficits and Abeta deposits in transgenic APP+PS1 mice. *Neurobiol Aging* **22**, 377-385 (2001).
67. Irizarry, M. C., McNamara, M., Fedorchak, K., Hsiao, K. & Hyman, B. T. APPSw transgenic mice develop age-related A beta deposits and neuropil abnormalities, but no neuronal loss in CA1. *J Neuropathol Exp Neurol* **56**, 965-973 (1997).
68. Kawarabayashi, T. et al. Age-dependent changes in brain, CSF, and plasma amyloid (beta) protein in the Tg2576 transgenic mouse model of Alzheimer's disease. *J Neurosci* **21**, 372-381 (2001).
69. King, D. L. & Arendash, G. W. Maintained synaptophysin immunoreactivity in Tg2576 transgenic mice during aging: correlations with cognitive impairment. *Brain Res* **926**, 58-68 (2002).
70. King, D. L. & Arendash, G. W. Behavioral characterization of the Tg2576 transgenic model of Alzheimer's disease through 19 months. *Physiol Behav* **75**, 627-642 (2002).
71. King, D. L. et al. Progressive and gender-dependent cognitive impairment in the APP(SW) transgenic mouse model for Alzheimer's disease. *Behav Brain Res* **103**, 145-162 (1999).
72. Lannfelt, L. et al. Amyloid precursor protein mutation causes Alzheimer's disease in a Swedish family. *Neurosci Lett* **168**, 254-256 (1994).
73. Kalaria, R. N. et al. Abundance of the longer A beta 42 in neocortical and cerebrovascular amyloid beta deposits in Swedish familial Alzheimer's disease and Down's syndrome. *Neuroreport* **7**, 1377-1381 (1996).
74. Hsiao, K. et al. Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. *Science* **274**, 99-102 (1996).
75. Pompl, P. N., Mullan, M. J., Bjugstad, K. & Arendash, G. W. Adaptation of the circular platform spatial memory task for mice: use in detecting cognitive impairment in the APP(SW) transgenic mouse model for Alzheimer's disease. *J Neurosci Methods* **87**, 87-95 (1999).
76. Westerman, M. A. et al. The relationship between Abeta and memory in the Tg2576 mouse model of Alzheimer's disease. *J Neurosci* **22**, 1858-1867 (2002).
77. Games, D. et al. Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. *Nature* **373**, 523-527 (1995).
78. Chen, G. et al. A learning deficit related to age and beta-amyloid plaques in a mouse model of Alzheimer's disease. *Nature* **408**, 975-979 (2000).
79. Hartman, R. E. et al. 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 (2005).
80. Oddo, S., Billings, L., Kesslak, J. P., Cribbs, D. H. & LaFerla, F. M. Abeta immunotherapy leads to clearance of early, but not late, hyperphosphorylated tau aggregates via the proteasome. *Neuron* **43**, 321-332 (2004).
81. Dodart, J. C. et al. Immunization reverses memory deficits without reducing brain Abeta burden in Alzheimer's disease model. *Nat Neurosci* **5**, 452-457 (2002).
82. Hartman, R. E. et al. Pomegranate juice decreases amyloid load and improves behavior in a mouse model of Alzheimer's disease. *Neurobiol Dis* **24**, 506-515 (2006).

83. Kotilinek, L. A. et al. Reversible memory loss in a mouse transgenic model of Alzheimer's disease. *J Neurosci* **22**, 6331-6335 (2002).
84. Wilcock, D. M., Jantzen, P. T., Li, Q., Morgan, D. & Gordon, M. N. Amyloid-beta vaccination, but not nitro-nonsteroidal anti-inflammatory drug treatment, increases vascular amyloid and microhemorrhage while both reduce parenchymal amyloid. *Neuroscience* **144**, 950-960 (2007).
85. Hardy, J. An 'anatomical cascade hypothesis' for Alzheimer's disease. *Trends Neurosci* **15**, 200-201 (1992).
86. Hardy, J. & Allsop, D. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. *Trends Pharmacol Sci* **12**, 383-388 (1991).
87. Hardy, J. A. & Higgins, G. A. Alzheimer's disease: the amyloid cascade hypothesis. *Science* **256**, 184-185 (1992).
88. Kamboh, M. I. Apolipoprotein E polymorphism and susceptibility to Alzheimer's disease. *Hum Biol* **67**, 195-215 (1995).
89. Katzman, R. Apolipoprotein E and Alzheimer's disease. *Curr Opin Neurobiol* **4**, 703-707 (1994).
90. Pericak-Vance, M. A. & Haines, J. L. Genetic susceptibility to Alzheimer disease. *Trends Genet* **11**, 504-508 (1995).
91. Poirier, J., Minnich, A. & Davignon, J. Apolipoprotein E, synaptic plasticity and Alzheimer's disease. *Ann Med* **27**, 663-670 (1995).
92. Roses, A. D. & Saunders, A. M. APOE is a major susceptibility gene for Alzheimer's disease. *Curr Opin Biotechnol* **5**, 663-667 (1994).
93. Coon, K. D. et al. A high-density whole-genome association study reveals that APOE is the major susceptibility gene for sporadic late-onset Alzheimer's disease. *J Clin Psychiatry* **68**, 613-618 (2007).
94. Corder, E. H. et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* **261**, 921-923 (1993).
95. Saunders, A. M. et al. Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology* **43**, 1467-1472 (1993).
96. Strittmatter, W. J. et al. 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 (1993).
97. Hartman, R. E. et al. Behavioral phenotyping of GFAP-apoE3 and -apoE4 transgenic mice: apoE4 mice show profound working memory impairments in the absence of Alzheimer's-like neuropathology. *Exp Neurol* **170**, 326-344 (2001).
98. Raber, J. et al. Isoform-specific effects of human apolipoprotein E on brain function revealed in ApoE knockout mice: increased susceptibility of females. *Proc Natl Acad Sci U S A* **95**, 10914-10919 (1998).
99. Kida, E. et al. Complete cerebral ischemia with short-term survival in rat induced by cardiac arrest. II. Extracellular and intracellular accumulation of apolipoproteins E and J in the brain. *Brain Res* **674**, 341-346 (1995).
100. Pluta, R. Experimental model of neuropathological changes characteristic for Alzheimer's disease. *Folia Neuropathol* **35**, 94-98 (1997).
101. Pluta, R. The role of apolipoprotein E in the deposition of beta-amyloid peptide during ischemia-reperfusion brain injury. A model of early Alzheimer's disease. *Ann N Y Acad Sci* **903**, 324-334 (2000).

102. Pluta, R., Barcikowska, M., Debicki, G., Ryba, M. & Januszewski, S. Changes in amyloid precursor protein and apolipoprotein E immunoreactivity following ischemic brain injury in rat with long-term survival: influence of idebenone treatment. *Neurosci Lett* **232**, 95-98 (1997).
103. Thal, D. R. et al. Apolipoprotein E co-localizes with newly formed amyloid beta-protein (Abeta) deposits lacking immunoreactivity against N-terminal epitopes of Abeta in a genotype-dependent manner. *Acta Neuropathol (Berl)* **110**, 459-471 (2005).
104. Wahrle, S. E. et al. Deletion of Abca1 increases Abeta deposition in the PDAPP transgenic mouse model of Alzheimer disease. *J Biol Chem* **280**, 43236-43242 (2005).
105. Bales, K. R. et al. Apolipoprotein E is essential for amyloid deposition in the APP(V717F) transgenic mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A* **96**, 15233-15238 (1999).
106. Bales, K. R., Dodart, J. C., DeMattos, R. B., Holtzman, D. M. & Paul, S. M. Apolipoprotein E, amyloid, and Alzheimer disease. *Mol Interv* **2**, 363-75, 339 (2002).
107. Fagan, A. M. et al. Human and murine ApoE markedly alters A beta metabolism before and after plaque formation in a mouse model of Alzheimer's disease. *Neurobiol Dis* **9**, 305-318 (2002).
108. Holtzman, D. M. et al. Apolipoprotein E isoform-dependent amyloid deposition and neuritic degeneration in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A* **97**, 2892-2897 (2000).
109. Holtzman, D. M. et al. Apolipoprotein E facilitates neuritic and cerebrovascular plaque formation in an Alzheimer's disease model. *Ann Neurol* **47**, 739-747 (2000).
110. Wahrle, S. E. et al. Apolipoprotein E levels in cerebrospinal fluid and the effects of ABCA1 polymorphisms. *Mol Neurodegener* **2**, 7 (2007).
111. Nilsson, L. N. et al. Cognitive impairment in PDAPP mice depends on ApoE and ACT-catalyzed amyloid formation. *Neurobiol Aging* **25**, 1153-1167 (2004).
112. Laskowitz, D. T., Fillit, H., Yeung, N., Toku, K. & Vitek, M. P. Apolipoprotein E-derived peptides reduce CNS inflammation: implications for therapy of neurological disease. *Acta Neurol Scand Suppl* **185**, 15-20 (2006).
113. Laskowitz, D. T. et al. Downregulation of microglial activation by apolipoprotein E and apoE-mimetic peptides. *Exp Neurol* **167**, 74-85 (2001).
114. Barger, S. W. & Harmon, A. D. Microglial activation by Alzheimer amyloid precursor protein and modulation by apolipoprotein E. *Nature* **388**, 878-881 (1997).
115. Mazur-Kolecka, B., Dickson, D. & Frackowiak, J. Induction of vascular amyloidosis-beta by oxidative stress depends on APOE genotype. *Neurobiol Aging* **27**, 804-814 (2006).
116. Mazur-Kolecka, B., Frackowiak, J., Kowal, D., Krzeslowska, J. & Dickson, D. Oxidative protein damage in cells engaged in beta-amyloidosis is related to apoE genotype. *Neuroreport* **13**, 465-468 (2002).
117. Mazur-Kolecka, B. et al. Apolipoprotein E alters metabolism of AbetaPP in cells engaged in beta-amyloidosis. *J Neuropathol Exp Neurol* **58**, 288-295 (1999).
118. Mazur-Kolecka, B., Kowal, D., Sukontasup, T., Dickson, D. & Frackowiak, J. The effect of oxidative stress on amyloid precursor protein processing in cells engaged

in beta-amyloidosis is related to apolipoprotein E genotype. *Acta Neuropathol (Berl)* **108**, 287-294 (2004).
119. Wu, C. W. et al. Brain region-dependent increases in beta-amyloid and apolipoprotein E levels in hypercholesterolemic rabbits. *J Neural Transm* **110**, 641-649 (2003).
120. Refolo, L. M. et al. Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model. *Neurobiol Dis* **7**, 321-331 (2000).
121. Cordy, J. M., Hussain, I., Dingwall, C., Hooper, N. M. & Turner, A. J. Exclusively targeting beta-secretase to lipid rafts by GPI-anchor addition up-regulates beta-site processing of the amyloid precursor protein. *Proc Natl Acad Sci U S A* **100**, 11735-11740 (2003).
122. Riddell, D. R., Christie, G., Hussain, I. & Dingwall, C. Compartmentalization of beta-secretase (Asp2) into low-buoyant density, noncaveolar lipid rafts. *Curr Biol* **11**, 1288-1293 (2001).
123. Vetrivel, K. S. et al. Spatial segregation of gamma-secretase and substrates in distinct membrane domains. *J Biol Chem* **280**, 25892-25900 (2005).
124. Vetrivel, K. S. et al. Association of gamma-secretase with lipid rafts in post-Golgi and endosome membranes. *J Biol Chem* **279**, 44945-44954 (2004).
125. Kojro, E., Gimpl, G., Lammich, S., Marz, W. & Fahrenholz, F. Low cholesterol stimulates the nonamyloidogenic pathway by its effect on the alpha -secretase ADAM 10. *Proc Natl Acad Sci U S A* **98**, 5815-5820 (2001).
126. Henderson, S. T. High carbohydrate diets and Alzheimer's disease. *Med Hypotheses* **62**, 689-700 (2004).
127. Calon, F. et al. Docosahexaenoic acid protects from dendritic pathology in an Alzheimer's disease mouse model. *Neuron* **43**, 633-645 (2004).
128. Cole, G. M. & Frautschy, S. A. Docosahexaenoic acid protects from amyloid and dendritic pathology in an Alzheimer's disease mouse model. *Nutr Health* **18**, 249-259 (2006).
129. Lim, G. P. et al. 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 (2005).
130. Ringman, J. M., Frautschy, S. A., Cole, G. M., Masterman, D. L. & Cummings, J. L. A potential role of the curry spice curcumin in Alzheimer's disease. *Curr Alzheimer Res* **2**, 131-136 (2005).
131. Yang, F. et al. Curcumin inhibits formation of amyloid beta oligomers and fibrils, binds plaques, and reduces amyloid in vivo. *J Biol Chem* **280**, 5892-5901 (2005).
132. Mayeux, R. et al. Synergistic effects of traumatic head injury and apolipoprotein-epsilon 4 in patients with Alzheimer's disease. *Neurology* **45**, 555-557 (1995).
133. Mayeux, R. et al. Genetic susceptibility and head injury as risk factors for Alzheimer's disease among community-dwelling elderly persons and their first-degree relatives. *Ann Neurol* **33**, 494-501 (1993).
134. Plassman, B. L. et al. Documented head injury in early adulthood and risk of Alzheimer's disease and other dementias. *Neurology* **55**, 1158-1166 (2000).
135. Clinton, J., Ambler, M. W. & Roberts, G. W. Post-traumatic Alzheimer's disease: preponderance of a single plaque type. *Neuropathol Appl Neurobiol* **17**, 69-74 (1991).

136. Gentleman, S. M. et al. A beta 42 is the predominant form of amyloid beta-protein in the brains of short-term survivors of head injury. *Neuroreport* **8**, 1519-1522 (1997).
137. Graham, D. I., Gentleman, S. M., Lynch, A. & Roberts, G. W. Distribution of beta-amyloid protein in the brain following severe head injury. *Neuropathol Appl Neurobiol* **21**, 27-34 (1995).
138. Roberts, G. W. et al. Beta amyloid protein deposition in the brain after severe head injury: implications for the pathogenesis of Alzheimer's disease. *J Neurol Neurosurg Psychiatry* **57**, 419-425 (1994).
139. Allsop, D., Haga, S., Bruton, C., Ishii, T. & Roberts, G. W. Neurofibrillary tangles in some cases of dementia pugilistica share antigens with amyloid beta-protein of Alzheimer's disease. *Am J Pathol* **136**, 255-260 (1990).
140. Jordan, B. D. Chronic traumatic brain injury associated with boxing. *Semin Neurol* **20**, 179-185 (2000).
141. Jordan, S. E., Green, G. A., Galanty, H. L., Mandelbaum, B. R. & Jabour, B. A. Acute and chronic brain injury in United States National Team soccer players. *Am J Sports Med* **24**, 205-210 (1996).
142. Roberts, G. W., Allsop, D. & Bruton, C. The occult aftermath of boxing. *J Neurol Neurosurg Psychiatry* **53**, 373-378 (1990).
143. Rudelli, R., Strom, J. O., Welch, P. T. & Ambler, M. W. Posttraumatic premature Alzheimer's disease. Neuropathologic findings and pathogenetic considerations. *Arch Neurol* **39**, 570-575 (1982).
144. Tokuda, T., Ikeda, S., Yanagisawa, N., Ihara, Y. & Glenner, G. G. Re-examination of ex-boxers' brains using immunohistochemistry with antibodies to amyloid beta-protein and tau protein. *Acta Neuropathol (Berl)* **82**, 280-285 (1991).
145. Toth, C., McNeil, S. & Feasby, T. Central nervous system injuries in sport and recreation: a systematic review. *Sports Med* **35**, 685-715 (2005).
146. Emmerling, M. R. et al. Traumatic brain injury elevates the Alzheimer's amyloid peptide A beta 42 in human CSF. A possible role for nerve cell injury. *Ann N Y Acad Sci* **903**, 118-122 (2000).
147. Franz, G. et al. Amyloid beta 1-42 and tau in cerebrospinal fluid after severe traumatic brain injury. *Neurology* **60**, 1457-1461 (2003).
148. Olsson, A. et al. Marked increase of beta-amyloid(1-42) and amyloid precursor protein in ventricular cerebrospinal fluid after severe traumatic brain injury. *J Neurol* **251**, 870-876 (2004).
149. Raby, C. A. et al. Traumatic brain injury increases beta-amyloid peptide 1-42 in cerebrospinal fluid. *J Neurochem* **71**, 2505-2509 (1998).
150. Attems, J. & Jellinger, K. A. Only cerebral capillary amyloid angiopathy correlates with Alzheimer pathology--a pilot study. *Acta Neuropathol (Berl)* **107**, 83-90 (2004).
151. Attems, J., Jellinger, K. A. & Lintner, F. Alzheimer's disease pathology influences severity and topographical distribution of cerebral amyloid angiopathy. *Acta Neuropathol (Berl)* **110**, 222-231 (2005).
152. Attems, J., Lintner, F. & Jellinger, K. A. Amyloid beta peptide 1-42 highly correlates with capillary cerebral amyloid angiopathy and Alzheimer disease pathology. *Acta Neuropathol (Berl)* **107**, 283-291 (2004).

153. Attems, J., Quass, M., Jellinger, K. A. & Lintner, F. Topographical distribution of cerebral amyloid angiopathy and its effect on cognitive decline are influenced by Alzheimer disease pathology. *J Neurol Sci* **257**, 49-55 (2007).
154. Jellinger, K. A. & Attems, J. Prevalence and pathogenic role of cerebrovascular lesions in Alzheimer disease. *J Neurol Sci* **229-230**, 37-41 (2005).
155. Jellinger, K. A. & Attems, J. Prevalence and impact of cerebrovascular pathology in Alzheimer's disease and parkinsonism. *Acta Neurol Scand* **114**, 38-46 (2006).
156. Gouras, G. K. et al. Increased apolipoprotein E epsilon 4 in epilepsy with senile plaques. *Ann Neurol* **41**, 402-404 (1997).
157. Szczygielski, J. et al. Traumatic brain injury: cause or risk of Alzheimer's disease? A review of experimental studies. *J Neural Transm* **112**, 1547-1564 (2005).
158. Otsuka, N., Tomonaga, M. & Ikeda, K. Rapid appearance of beta-amyloid precursor protein immunoreactivity in damaged axons and reactive glial cells in rat brain following needle stab injury. *Brain Res* **568**, 335-338 (1991).
159. Blasko, I. et al. Experimental traumatic brain injury in rats stimulates the expression, production and activity of Alzheimer's disease beta-secretase (BACE-1). *J Neural Transm* **111**, 523-536 (2004).
160. Hamberger, A. et al. Redistribution of neurofilaments and accumulation of beta-amyloid protein after brain injury by rotational acceleration of the head. *J Neurotrauma* **20**, 169-178 (2003).
161. Hoshino, S. et al. Emergence of immunoreactivities for phosphorylated tau and amyloid-beta protein in chronic stage of fluid percussion injury in rat brain. *Neuroreport* **9**, 1879-1883 (1998).
162. Iwata, A., Chen, X. H., McIntosh, T. K., Browne, K. D. & Smith, D. H. Long-term accumulation of amyloid-beta in axons following brain trauma without persistent upregulation of amyloid precursor protein genes. *J Neuropathol Exp Neurol* **61**, 1056-1068 (2002).
163. Smith, D. H. et al. Brain trauma induces massive hippocampal neuron death linked to a surge in beta-amyloid levels in mice overexpressing mutant amyloid precursor protein. *Am J Pathol* **153**, 1005-1010 (1998).
164. Conte, V. et al. Vitamin E reduces amyloidosis and improves cognitive function in Tg2576 mice following repetitive concussive brain injury. *J Neurochem* **90**, 758-764 (2004).
165. Uryu, K. et al. Repetitive mild brain trauma accelerates Abeta deposition, lipid peroxidation, and cognitive impairment in a transgenic mouse model of Alzheimer amyloidosis. *J Neurosci* **22**, 446-454 (2002).
166. Koob, A. O. & Borgens, R. B. Polyethylene glycol treatment after traumatic brain injury reduces beta-amyloid precursor protein accumulation in degenerating axons. *J Neurosci Res* **83**, 1558-1563 (2006).
167. Koob, A. O., Duerstock, B. S., Babbs, C. F., Sun, Y. & Borgens, R. B. Intravenous polyethylene glycol inhibits the loss of cerebral cells after brain injury. *J Neurotrauma* **22**, 1092-1111 (2005).
168. Lewen, A., Li, G. L., Nilsson, P., Olsson, Y. & Hillered, L. Traumatic brain injury in rat produces changes of beta-amyloid precursor protein immunoreactivity. *Neuroreport* **6**, 357-360 (1995).

169. Lewen, A., Li, G. L., Olsson, Y. & Hillered, L. Changes in microtubule-associated protein 2 and amyloid precursor protein immunoreactivity following traumatic brain injury in rat: influence of MK-801 treatment. *Brain Res* **719**, 161-171 (1996).
170. Brody, D. L. & Holtzman, D. M. Morris water maze search strategy analysis in PDAPP mice before and after experimental traumatic brain injury. *Exp Neurol* **197**, 330-340 (2006).
171. Brody, D. L. et al. Electromagnetic controlled cortical impact device for precise, graded experimental traumatic brain injury. *J Neurotrauma* **24**, 657-673 (2007).
172. Mac Donald, C. L. et al. Detection of traumatic axonal injury with diffusion tensor imaging in a mouse model of traumatic brain injury. *Exp Neurol* **205**, 116-131 (2007).
173. Ezra, Y. et al. Apolipoprotein E4 decreases whereas apolipoprotein E3 increases the level of secreted amyloid precursor protein after closed head injury. *Neuroscience* **121**, 315-325 (2003).
174. Thornton, E., Vink, R., Blumbergs, P. C. & Van Den Heuvel, C. Soluble amyloid precursor protein alpha reduces neuronal injury and improves functional outcome following diffuse traumatic brain injury in rats. *Brain Res* **1094**, 38-46 (2006).
175. Ben Simon, G. J., Hovda, D. A., Harris, N. G., Gomez-Pinilla, F. & Goldberg, R. A. Traumatic brain injury induced neuroprotection of retinal ganglion cells to optic nerve crush. *J Neurotrauma* **23**, 1072-1082 (2006).
176. Imai, H. et al. Specific expression of the cell cycle regulation proteins, GADD34 and PCNA, in the peri-infarct zone after focal cerebral ischaemia in the rat. *Eur J Neurosci* **15**, 1929-1936 (2002).
177. Pluta, R., Barcikowska, M., Mossakowski, M. J. & Zelman, I. Cerebral accumulation of beta-amyloid following ischemic brain injury with long-term survival. *Acta Neurochir Suppl* **71**, 206-208 (1998).
178. Pluta, R. et al. Complete cerebral ischemia with short-term survival in rats induced by cardiac arrest. I. Extracellular accumulation of Alzheimer's beta-amyloid protein precursor in the brain. *Brain Res* **649**, 323-328 (1994).
179. Irving, E. A., Bentley, D. L. & Parsons, A. A. Assessment of white matter injury following prolonged focal cerebral ischaemia in the rat. *Acta Neuropathol (Berl)* **102**, 627-635 (2001).
180. Abe, K., Tanzi, R. E. & Kogure, K. Selective induction of Kunitz-type protease inhibitor domain-containing amyloid precursor protein mRNA after persistent focal ischemia in rat cerebral cortex. *Neurosci Lett* **125**, 172-174 (1991).
181. Koistinaho, J. & Hokfelt, T. Altered gene expression in brain ischemia. *Neuroreport* **8**, i-viii (1997).
182. Koistinaho, J., Pyykonen, I., Keinanen, R. & Hokfelt, T. Expression of beta-amyloid precursor protein mRNAs following transient focal ischaemia. *Neuroreport* **7**, 2727-2731 (1996).
183. Heurteaux, C., Bertaina, V., Widmann, C. & Lazdunski, M. K+ channel openers prevent global ischemia-induced expression of c-fos, c-jun, heat shock protein, and amyloid beta-protein precursor genes and neuronal death in rat hippocampus. *Proc Natl Acad Sci U S A* **90**, 9431-9435 (1993).
184. Kim, H. S. et al. Post-ischemic changes in the expression of Alzheimer's APP isoforms in rat cerebral cortex. *Neuroreport* **9**, 533-537 (1998).

185. Scott, J. N., Parhad, I. M. & Clark, A. W. Beta-amyloid precursor protein gene is differentially expressed in axotomized sensory and motor systems. *Brain Res Mol Brain Res* **10**, 315-325 (1991).
186. Komori, N. et al. Reversible ischemia increases levels of Alzheimer amyloid protein precursor without increasing levels of mRNA in the rabbit spinal cord. *Brain Res Mol Brain Res* **49**, 103-112 (1997).
187. Chen, G. J., Xu, J., Lahousse, S. A., Caggiano, N. L. & de la Monte, S. M. Transient hypoxia causes Alzheimer-type molecular and biochemical abnormalities in cortical neurons: potential strategies for neuroprotection. *J Alzheimers Dis* **5**, 209-228 (2003).
188. Olney, J. W. Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. *Science* **164**, 719-721 (1969).
189. Panegyres, P. K. The effects of excitotoxicity on the expression of the amyloid precursor protein gene in the brain and its modulation by neuroprotective agents. *J Neural Transm* **105**, 463-478 (1998).
190. Liberman, J. N., Stewart, W. F., Wesnes, K. & Troncoso, J. Apolipoprotein E epsilon 4 and short-term recovery from predominantly mild brain injury. *Neurology* **58**, 1038-1044 (2002).
191. Friedman, G. et al. Apolipoprotein E-epsilon4 genotype predicts a poor outcome in survivors of traumatic brain injury. *Neurology* **52**, 244-248 (1999).
192. Teasdale, G. M., Nicoll, J. A., Murray, G. & Fiddes, M. Association of apolipoprotein E polymorphism with outcome after head injury. *Lancet* **350**, 1069-1071 (1997).
193. Crawford, F. C. et al. APOE genotype influences acquisition and recall following traumatic brain injury. *Neurology* **58**, 1115-1118 (2002).
194. Lichtman, S. W., Seliger, G., Tycko, B. & Marder, K. Apolipoprotein E and functional recovery from brain injury following postacute rehabilitation. *Neurology* **55**, 1536-1539 (2000).
195. Jordan, B. D. et al. Apolipoprotein E epsilon4 associated with chronic traumatic brain injury in boxing. *JAMA* **278**, 136-140 (1997).
196. Kutner, K. C., Erlanger, D. M., Tsai, J., Jordan, B. & Relkin, N. R. Lower cognitive performance of older football players possessing apolipoprotein E epsilon4. *Neurosurgery* **47**, 651-7; discussion 657-8 (2000).
197. Tang, M. X. et al. Effect of age, ethnicity, and head injury on the association between APOE genotypes and Alzheimer's disease. *Ann N Y Acad Sci* **802**, 6-15 (1996).
198. Roberts, G. W., Gentleman, S. M., Lynch, A. & Graham, D. I. beta A4 amyloid protein deposition in brain after head trauma. *Lancet* **338**, 1422-1423 (1991).
199. Macfarlane, D. P., Nicoll, J. A., Smith, C. & Graham, D. I. APOE epsilon4 allele and amyloid beta-protein deposition in long term survivors of head injury. *Neuroreport* **10**, 3945-3948 (1999).
200. Nicoll, J. A., Roberts, G. W. & Graham, D. I. Apolipoprotein E epsilon 4 allele is associated with deposition of amyloid beta-protein following head injury. *Nat Med* **1**, 135-137 (1995).
201. Nicoll, J. A., Roberts, G. W. & Graham, D. I. Amyloid beta-protein, APOE genotype and head injury. *Ann N Y Acad Sci* **777**, 271-275 (1996).

202. Sabo, T. et al. Susceptibility of transgenic mice expressing human apolipoprotein E to closed head injury: the allele E3 is neuroprotective whereas E4 increases fatalities. *Neuroscience* **101**, 879-884 (2000).
203. Hartman, R. E. et al. Apolipoprotein E4 influences amyloid deposition but not cell loss after traumatic brain injury in a mouse model of Alzheimer's disease. *J Neurosci* **22**, 10083-10087 (2002).
204. Buttini, M. et al. Expression of human apolipoprotein E3 or E4 in the brains of Apoe-/- mice: isoform-specific effects on neurodegeneration. *J Neurosci* **19**, 4867-4880 (1999).
205. Chen, Y., Lomnitski, L., Michaelson, D. M. & Shohami, E. Motor and cognitive deficits in apolipoprotein E-deficient mice after closed head injury. *Neuroscience* **80**, 1255-1262 (1997).
206. Fagan, A. M. et al. Evidence for normal aging of the septo-hippocampal cholinergic system in apoE (-/-) mice but impaired clearance of axonal degeneration products following injury. *Exp Neurol* **151**, 314-325 (1998).
207. Genis, L., Chen, Y., Shohami, E. & Michaelson, D. M. Tau hyperphosphorylation in apolipoprotein E-deficient and control mice after closed head injury. *J Neurosci Res* **60**, 559-564 (2000).
208. Sheng, H. et al. Apolipoprotein E isoform-specific differences in outcome from focal ischemia in transgenic mice. *J Cereb Blood Flow Metab* **18**, 361-366 (1998).
209. Stone, D. J., Rozovsky, I., Morgan, T. E., Anderson, C. P. & Finch, C. E. Increased synaptic sprouting in response to estrogen via an apolipoprotein E-dependent mechanism: implications for Alzheimer's disease. *J Neurosci* **18**, 3180-3185 (1998).
210. Holtzman, D. M. Role of apoe/Abeta interactions in the pathogenesis of Alzheimer's disease and cerebral amyloid angiopathy. *J Mol Neurosci* **17**, 147-155 (2001).
211. Wisniewski, T., Ghiso, J. & Frangione, B. Biology of A beta amyloid in Alzheimer's disease. *Neurobiol Dis* **4**, 313-328 (1997).
212. Bales, K. R. et al. Lack of apolipoprotein E dramatically reduces amyloid beta-peptide deposition. *Nat Genet* **17(3)**, 263-264 (1997).
213. Teter, B. ApoE-dependent plasticity in Alzheimer's disease. *J Mol Neurosci* **23**, 167-179 (2004).
214. Erkinjuntti, T., Partinen, M., Sulkava, R., Palomaki, H. & Tilvis, R. Snoring and dementia. *Age Ageing* **16**, 305-310 (1987).
215. Mossakowski, M. J., Lossinsky, A. S., Pluta, R. & Wisniewski, H. M. Abnormalities of the blood-brain barrier in global cerebral ischemia in rats due to experimental cardiac arrest. *Acta Neurochir Suppl (Wien)* **60**, 274-276 (1994).
216. Pluta, R. Blood-brain barrier dysfunction and amyloid precursor protein accumulation in microvascular compartment following ischemia-reperfusion brain injury with 1-year survival. *Acta Neurochir Suppl* **86**, 117-122 (2003).
217. Pluta, R. Pathological opening of the blood-brain barrier to horseradish peroxidase and amyloid precursor protein following ischemia-reperfusion brain injury. *Chemotherapy* **51**, 223-226 (2005).
218. Pluta, R. Is the ischemic blood-brain barrier insufficiency responsible for full-blown Alzheimer's disease? *Neurol Res* **28**, 665-671 (2006).

219. Pluta, R. Role of ischemic blood-brain barrier on amyloid plaques development in Alzheimer's disease brain. *Curr Neurovasc Res* **4**, 121-129 (2007).
220. Pluta, R., Barcikowska, M., Januszewski, S., Misicka, A. & Lipkowski, A. W. Evidence of blood-brain barrier permeability/leakage for circulating human Alzheimer's beta-amyloid-(1-42)-peptide. *Neuroreport* **7**, 1261-1265 (1996).
221. Pluta, R. et al. Ischemic rats as a model in the study of the neurobiological role of human beta-amyloid peptide. Time-dependent disappearing diffuse amyloid plaques in brain. *Neuroreport* **10**, 3615-3619 (1999).
222. Pluta, R., Lossinsky, A. S., Wisniewski, H. M. & Mossakowski, M. J. Early blood-brain barrier changes in the rat following transient complete cerebral ischemia induced by cardiac arrest. *Brain Res* **633**, 41-52 (1994).
223. Pluta, R. et al. Possible reverse transport of beta-amyloid peptide across the blood-brain barrier. *Acta Neurochir Suppl* **76**, 73-77 (2000).
224. Pluta, R., Misicka, A., Januszewski, S., Barcikowska, M. & Lipkowski, A. W. Transport of human beta-amyloid peptide through the rat blood-brain barrier after global cerebral ischemia. *Acta Neurochir Suppl* **70**, 247-249 (1997).
225. Pluta, R., Ulamek, M. & Januszewski, S. Micro-blood-brain barrier openings and cytotoxic fragments of amyloid precursor protein accumulation in white matter after ischemic brain injury in long-lived rats. *Acta Neurochir Suppl* **96**, 267-271 (2006).
226. Ciallella, J. R. et al. Changes in expression of amyloid precursor protein and interleukin-1beta after experimental traumatic brain injury in rats. *J Neurotrauma* **19**, 1555-1567 (2002).
227. Gentleman, S. M., Nash, M. J., Sweeting, C. J., Graham, D. I. & Roberts, G. W. Beta-amyloid precursor protein (beta APP) as a marker for axonal injury after head injury. *Neurosci Lett* **160**, 139-144 (1993).
228. Graham, D. I. et al. Altered beta-APP metabolism after head injury and its relationship to the aetiology of Alzheimer's disease. *Acta Neurochir Suppl* **66**, 96-102 (1996).
229. Griffin, W. S. et al. Microglial interleukin-1 alpha expression in human head injury: correlations with neuronal and neuritic beta-amyloid precursor protein expression. *Neurosci Lett* **176**, 133-136 (1994).
230. Love, R. Calorie restriction may be neuroprotective in AD and PD. *Lancet Neurol* **4**, 84 (2005).
231. Wang, J. et al. Caloric restriction attenuates beta-amyloid neuropathology in a mouse model of Alzheimer's disease. *FASEB J* **19**, 659-661 (2005).
232. Patel, N. V. et al. Caloric restriction attenuates Abeta-deposition in Alzheimer transgenic models. *Neurobiol Aging* **26**, 995-1000 (2005).
233. Mattson, M. P. Existing data suggest that Alzheimer's disease is preventable. *Ann N Y Acad Sci* **924**, 153-159 (2000).
234. Morris, M. C. et al. Dietary niacin and the risk of incident Alzheimer's disease and of cognitive decline. *J Neurol Neurosurg Psychiatry* **75**, 1093-1099 (2004).
235. Pope, S. K., Shue, V. M. & Beck, C. Will a healthy lifestyle help prevent Alzheimer's disease? *Annu Rev Public Health* **24**, 111-132 (2003).
236. Kostrzewa, R. M. & Segura-Aguilar, J. Novel mechanisms and approaches in the study of neurodegeneration and neuroprotection. a review. *Neurotox Res* **5**, 375-383 (2003).

237. Polidori, M. C. Antioxidant micronutrients in the prevention of age-related diseases. *J Postgrad Med* **49**, 229-235 (2003).
238. Morris, M. C. et al. Relation of the tocopherol forms to incident Alzheimer disease and to cognitive change. *Am J Clin Nutr* **81**, 508-514 (2005).
239. Sung, S. et al. Early vitamin E supplementation in young but not aged mice reduces Abeta levels and amyloid deposition in a transgenic model of Alzheimer's disease. *FASEB J* **18**, 323-325 (2004).
240. Aggarwal, B. B. & Shishodia, S. Suppression of the nuclear factor-kappaB activation pathway by spice-derived phytochemicals: reasoning for seasoning. *Ann N Y Acad Sci* **1030**, 434-441 (2004).
241. Joseph, J. A., Shukitt-Hale, B. & Casadesus, G. Reversing the deleterious effects of aging on neuronal communication and behavior: beneficial properties of fruit polyphenolic compounds. *Am J Clin Nutr* **81**, 313S-316S (2005).
242. Lim, G. P. et al. The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. *J Neurosci* **21**, 8370-8377 (2001).
243. Rezai-Zadeh, K. et al. Green tea epigallocatechin-3-gallate (EGCG) modulates amyloid precursor protein cleavage and reduces cerebral amyloidosis in Alzheimer transgenic mice. *J Neurosci* **25**, 8807-8814 (2005).
244. Ono, K., Hasegawa, K., Naiki, H. & Yamada, M. Anti-amyloidogenic activity of tannic acid and its activity to destabilize Alzheimer's beta-amyloid fibrils in vitro. *Biochim Biophys Acta* **1690**, 193-202 (2004).
245. Marambaud, P., Zhao, H. & Davies, P. Resveratrol promotes clearance of Alzheimer's disease amyloid-beta peptides. *J Biol Chem* **280**, 37377-37382 (2005).
246. Seeram, N. P., Adams, L. S., Hardy, M. L. & Heber, D. Total cranberry extract versus its phytochemical constituents: antiproliferative and synergistic effects against human tumor cell lines. *J Agric Food Chem* **52**, 2512-2517 (2004).
247. Seeram, N. P. et al. In vitro antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. *J Nutr Biochem* **16**, 360-367 (2005).
248. Weinreb, O., Mandel, S., Amit, T. & Youdim, M. B. Neurological mechanisms of green tea polyphenols in Alzheimer's and Parkinson's diseases. *J Nutr Biochem* **15**, 506-516 (2004).
249. Dai, Q., Borenstein, A. R., Wu, Y., Jackson, J. C. & Larson, E. B. Fruit and vegetable juices and Alzheimer's disease: the Kame Project. *Am J Med* **119**, 751-759 (2006).
250. Joseph, J. A., Shukitt-Hale, B., Casadesus, G. & Fisher, D. Oxidative stress and inflammation in brain aging: nutritional considerations. *Neurochem Res* **30**, 927-935 (2005).
251. Lahiri, D. K. Where the actions of environment (nutrition), gene and protein meet: beneficial role of fruit and vegetable juices in potentially delaying the onset of Alzheimer's disease. *J Alzheimers Dis* **10**, 359-61; discussion 363-4 (2006).
252. Lau, F. C., Shukitt-Hale, B. & Joseph, J. A. The beneficial effects of fruit polyphenols on brain aging. *Neurobiol Aging* **26 Suppl 1**, 128-132 (2005).
253. Joseph, J. A. et al. Blueberry supplementation enhances signaling and prevents behavioral deficits in an Alzheimer disease model. *Nutr Neurosci* **6**, 153-162 (2003).

254. Kelawala, N. S. & Ananthanarayan, L. Antioxidant activity of selected foodstuffs. *Int J Food Sci Nutr* **55**, 511-516 (2004).
255. Wang, R. F. et al. Bioactive compounds from the seeds of Punica granatum (pomegranate). *J Nat Prod* **67**, 2096-2098 (2004).
256. Loren, D. J., Seeram, N. P., Schulman, R. N. & Holtzman, D. M. Maternal dietary supplementation with pomegranate juice is neuroprotective in an animal model of neonatal hypoxic-ischemic brain injury. *Pediatr Res* **57**, 858-864 (2005).
257. Kwak, H. M. et al. beta-Secretase (BACE1) inhibitors from pomegranate (Punica granatum) husk. *Arch Pharm Res* **28**, 1328-1332 (2005).
258. Whitfield, J. F. Can statins put the brakes on Alzheimer's disease? *Expert Opin Investig Drugs* **15**, 1479-1485 (2006).
259. Grant, W. B. Dietary links to Alzheimer's disease: 1999 update. *J Alzheimers Dis* **1**, 197-201 (1999).
260. Grant, W. B. Fish consumption, cancer, and Alzheimer disease. *Am J Clin Nutr* **71**, 599-600 (2000).
261. Grant, W. B. Diet and risk of dementia: does fat matter? The Rotterdam Study. *Neurology* **60**, 2020-2021 (2003).
262. Horrocks, L. A. & Yeo, Y. K. Health benefits of docosahexaenoic acid (DHA). *Pharmacol Res* **40**, 211-225 (1999).
263. Peers, R. J. Alzheimer's disease and omega-3 fatty acids: hypothesis. *Med J Aust* **153**, 563-564 (1990).
264. Hashimoto, M., Hossain, S., Agdul, H. & Shido, O. Docosahexaenoic acid-induced amelioration on impairment of memory learning in amyloid beta-infused rats relates to the decreases of amyloid beta and cholesterol levels in detergent-insoluble membrane fractions. *Biochim Biophys Acta* **1738**, 91-98 (2005).
265. Hashimoto, M. et al. Chronic administration of docosahexaenoic acid ameliorates the impairment of spatial cognition learning ability in amyloid beta-infused rats. *J Nutr* **135**, 549-555 (2005).
266. Cirrito, J. R. & Holtzman, D. M. Amyloid beta and Alzheimer disease therapeutics: the devil may be in the details. *J Clin Invest* **112**, 321-323 (2003).
267. Eriksen, J. L. et al. NSAIDs and enantiomers of flurbiprofen target gamma-secretase and lower Abeta 42 in vivo. *J Clin Invest* **112**, 440-449 (2003).
268. Lim, G. P. et al. Ibuprofen effects on Alzheimer pathology and open field activity in APPsw transgenic mice. *Neurobiol Aging* **22**, 983-991 (2001).
269. Weggen, S. et al. Evidence that nonsteroidal anti-inflammatory drugs decrease amyloid beta 42 production by direct modulation of gamma-secretase activity. *J Biol Chem* **278**, 31831-31837 (2003).
270. Slack, B. E., Breu, J., Muchnicki, L. & Wurtman, R. J. Rapid stimulation of amyloid precursor protein release by epidermal growth factor: role of protein kinase C. *Biochem J* **327**, 245-249 (1997).
271. Cole, G. M. et al. NSAID and antioxidant prevention of Alzheimer's disease: lessons from in vitro and animal models. *Ann N Y Acad Sci* **1035**, 68-84 (2004).
272. Colciaghi, F. et al. Amyloid precursor protein metabolism is regulated toward alpha-secretase pathway by Ginkgo biloba extracts. *Neurobiol Dis* **16**, 454-460 (2004).

273. Hock, C. et al. Treatment with the selective muscarinic m1 agonist talsaclidine decreases cerebrospinal fluid levels of A beta 42 in patients with Alzheimer's disease. *Amyloid* **10**, 1-6 (2003).
274. Liskowsky, W. & Schliebs, R. Muscarinic acetylcholine receptor inhibition in transgenic Alzheimer-like Tg2576 mice by scopolamine favours the amyloidogenic route of processing of amyloid precursor protein. *Int J Dev Neurosci* **24**, 149-156 (2006).
275. Muller, D. M., Mendla, K., Farber, S. A. & Nitsch, R. M. Muscarinic M1 receptor agonists increase the secretion of the amyloid precursor protein ectodomain. *Life Sci* **60**, 985-991 (1997).
276. Rossner, S., Ueberham, U., Schliebs, R., Perez-Polo, J. R. & Bigl, V. The regulation of amyloid precursor protein metabolism by cholinergic mechanisms and neurotrophin receptor signaling. *Prog Neurobiol* **56**, 541-569 (1998).
277. Hussain, I. et al. Oral administration of a potent and selective non-peptidic BACE-1 inhibitor decreases beta-cleavage of amyloid precursor protein and amyloid-beta production in vivo. *J Neurochem* **100**, 802-809 (2007).
278. Xia, W. Amyloid inhibitors and Alzheimer's disease. *Curr Opin Investig Drugs* **4**, 55-59 (2003).
279. Ziani-Cherif, C., Mostefa-Kara, B. & Brixi-Gormat, F. Z. Gamma-secretase as a pharmacological target in Alzheimer disease research: when, why and how? *Curr Pharm Des* **12**, 4313-4335 (2006).
280. Best, J. D. et al. The novel gamma secretase inhibitor N-[cis-4-[(4-chlorophenyl)sulfonyl]-4-(2,5-difluorophenyl)cyclohexyl]-1,1,1-trifluoromethanesulfonamide (MRK-560) reduces amyloid plaque deposition without evidence of notch-related pathology in the Tg2576 mouse. *J Pharmacol Exp Ther* **320**, 552-558 (2007).
281. Sisodia, S. S., Kim, S. H. & Thinakaran, G. Function and dysfunction of the presenilins. *Am J Hum Genet* **65**, 7-12 (1999).
282. Thinakaran, G. & Parent, A. T. Identification of the role of presenilins beyond Alzheimer's disease. *Pharmacol Res* **50**, 411-418 (2004).
283. Turner, A. J., Fisk, L. & Nalivaeva, N. N. Targeting amyloid-degrading enzymes as therapeutic strategies in neurodegeneration. *Ann N Y Acad Sci* **1035**, 1-20 (2004).
284. Yan, P. et al. Matrix metalloproteinase-9 degrades amyloid-beta fibrils in vitro and compact plaques in situ. *J Biol Chem* **281**, 24566-24574 (2006).
285. Brendza, R. P. & Holtzman, D. M. Amyloid-beta immunotherapies in mice and men. *Alzheimer Dis Assoc Disord* **20**, 118-123 (2006).
286. Arendash, G. W. et al. Behavioral assessment of Alzheimer's transgenic mice following long-term Abeta vaccination: task specificity and correlations between Abeta deposition and spatial memory. *DNA Cell Biol* **20**, 737-744 (2001).
287. Holtzman, D. M., Bales, K. R., Paul, S. M. & DeMattos, R. B. Abeta immunization and anti-Abeta antibodies: potential therapies for the prevention and treatment of Alzheimer's disease. *Adv Drug Deliv Rev* **54**, 1603-1613 (2002).
288. Janus, C. et al. A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. *Nature* **408**, 979-982 (2000).
289. Morgan, D. et al. A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature* **408**, 982-985 (2000).

290. Schenk, D. et al. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* **400**, 173-177 (1999).
291. Nicoll, J. A. et al. Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report. *Nat Med* **9**, 448-452 (2003).
292. Bard, F. et al. 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 (2000).
293. DeMattos, R. B. et al. 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 (2001).
294. Wilcock, D. M. et al. Passive amyloid immunotherapy clears amyloid and transiently activates microglia in a transgenic mouse model of amyloid deposition. *J Neurosci* **24**, 6144-6151 (2004).
295. Buttini, M. et al. Beta-amyloid immunotherapy prevents synaptic degeneration in a mouse model of Alzheimer's disease. *J Neurosci* **25**, 9096-9101 (2005).
296. Lee, E. B. et al. Targeting amyloid-beta peptide (Abeta) oligomers by passive immunization with a conformation-selective monoclonal antibody improves learning and memory in Abeta precursor protein (APP) transgenic mice. *J Biol Chem* **281**, 4292-4299 (2006).
297. Bacskai, B. J. et al. Non-Fc-mediated mechanisms are involved in clearance of amyloid-beta in vivo by immunotherapy. *J Neurosci* **22**, 7873-7878 (2002).
298. Bard, F. et al. Epitope and isotype specificities of antibodies to beta -amyloid peptide for protection against Alzheimer's disease-like neuropathology. *Proc Natl Acad Sci U S A* **100**, 2023-2028 (2003).
299. Wilcock, D. M. et al. Intracranially administered anti-Abeta antibodies reduce beta-amyloid deposition by mechanisms both independent of and associated with microglial activation. *J Neurosci* **23**, 3745-3751 (2003).
300. Matsuoka, Y. et al. Novel therapeutic approach for the treatment of Alzheimer's disease by peripheral administration of agents with an affinity to beta-amyloid. *J Neurosci* **23**, 29-33 (2003).
301. Wilcock, D. M. et al. Passive immunotherapy against Abeta in aged APP-transgenic mice reverses cognitive deficits and depletes parenchymal amyloid deposits in spite of increased vascular amyloid and microhemorrhage. *J Neuroinflammation* **1**, 24 (2004).
302. Racke, M. M. et al. 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 (2005).
303. Fukuchi, K. et al. Anti-Abeta single-chain antibody delivery via adeno-associated virus for treatment of Alzheimer's disease. *Neurobiol Dis* **23**, 502-511 (2006).
304. Levites, Y. et al. Intracranial adeno-associated virus-mediated delivery of anti-pan amyloid beta, amyloid beta40, and amyloid beta42 single-chain variable fragments attenuates plaque pathology in amyloid precursor protein mice. *J Neurosci* **26**, 11923-11928 (2006).