Pathways towards and away from Alzheimer's disease

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Slowly but surely, Alzheimer's disease (AD) patients lose their memory and their cognitive abilities, and even their personalities may change dramatically. These changes are due to the progressive dysfunction and death of nerve cells that are responsible for the storage and processing of information. Although drugs can temporarily improve memory, at present there are no treatments that can stop or reverse the inexorable neurodegenerative process. But rapid progress towards understanding the cellular and molecular alterations that are responsible for the neuron's demise may soon help in developing effective preventative and therapeutic strategies.

Alzheimer's disease (AD) is a neurodegenerative disorder that currently affects nearly 2% of the population in industrialized countries; the risk of AD dramatically increases in individuals beyond the age of 70 and it is predicted that the incidence of AD will increase three-fold within the next 50 years (http://www.alz.org). Brain regions involved in learning and memory processes, including the temporal and frontal lobes, are reduced in size in AD patients as the result of degeneration of synapses and death of neurons (Fig. 1). Because there can be other causes of memory loss, definitive diagnosis of AD requires postmortem examination of the brain, which must contain sufficient numbers of "plaques" and "tangles" to qualify as affected by AD[1,2]. Plaques are extracellular deposits of fibrils and amorphous aggregates of amyloid β-peptide (Aβ); diffuse deposits of Aβ are also present in high amounts. Neurofibrillary tangles are intracellular fibrillar aggregates of the microtubule-associated protein tau that exhibit hyperphosphorylation and oxidative modifications. Plaques and tangles are present mainly in brain regions involved in learning and memory and emotional behaviours such as the entorhinal cortex, hippocampus, basal forebrain and amygdala. Brain regions with plaques typically exhibit reduced numbers of synapses, and neurites associated with the plaques are often damaged, which suggests that Aβ damages synapses and neurites. Neurons that use glutamate or acetylcholine as neurotransmitters appear to be particularly affected, but neurons that produce serotonin and norepinephrine are also damaged. This review focuses on the molecular and cellular abnormalities that occur in the brain in AD, how they result in synaptic dysfunction and cell death, and how they can be counteracted. Central to the disease is altered proteolytic processing of the amyloid precursor protein (APP) resulting in the production and aggregation of neurotoxic forms of Aβ. Neurons that degenerate in AD exhibit increased oxidative damage, impaired energy metabolism and perturbed cellular calcium homeostasis; Aβ appears to be an important instigator of these abnormalities. Genetic and environmental factors can determine one's risk for AD and an understanding of these risk factors and how they modify the amyloid cascade is leading to the development of interventions for the prevention and treatment of AD that range from changes in diet and lifestyle, to vaccines and drugs.

Pathways towards Alzheimer's disease

You cannot choose your parents

The genes passed on to you from your parents may or may not have a major role in determining whether you develop AD. The discoveries during the past 15 years of genetic aberrancies that either cause or increase the risk of AD heralded a rapid increase in knowledge of the molecular and cellular alterations responsible for neuronal degeneration and cognitive dysfunction in AD[3]. Once the amino-acid sequence of Aβ was determined, the gene encoding APP was cloned and localized to chromosome 21. DNA samples from pedigrees in which dominantly inherited AD occurred were then screened for mutations in APP, and several causative mutations were found[4]. Subsequent linkage analysis identified a region of chromosome 14 as the locus of a mutation(s) responsible for inherited AD

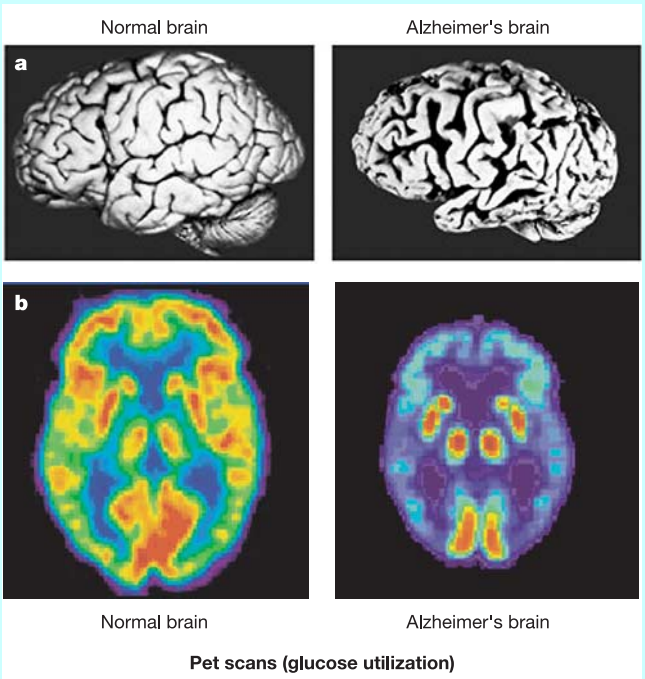

Figure 1 Alzheimer's disease results in shrinkage of brain regions involved in learning and memory which is correlated with major reductions in cellular energy metabolism in living patients. **a**, Compared with the brain of a healthy person, the brain of an Alzheimer's disease patient exhibits marked shrinkage of gyri in the temporal lobe (lower part of the brain) and frontal lobes (left part of the brain). **b**, Positron emission tomography (Pet) images showing glucose uptake (red and yellow indicate high levels of glucose uptake) in a living healthy person and a normal control subject. The Alzheimer's patient exhibits large decreases in energy metabolism in the frontal cortex (top of brain) and temporal lobes (sides of the brain).

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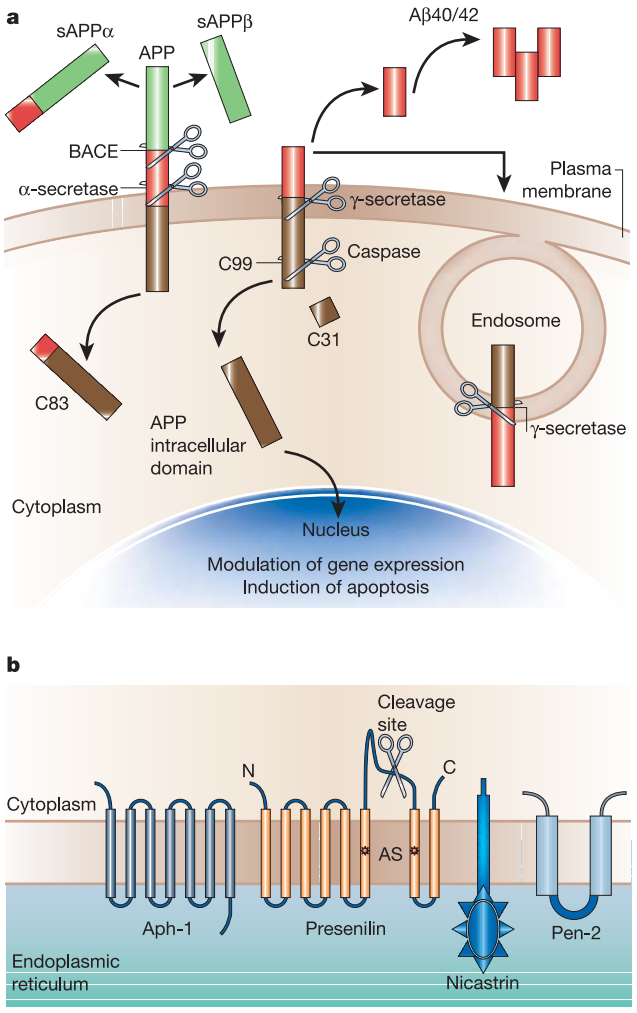
Box 1
APP cut down to size

APP is widely expressed in cells throughout the body where the amount produced is influenced by the developmental and physiological state of the cells. APP is an integral membrane protein with a single membrane-spanning domain, a large extracellular glycosylated N terminus and a shorter cytoplasmic C terminus—Aβ is located at the cell surface (or on the lumenal side of ER and Golgi membranes), with part of the peptide embedded in the membrane (Box 1 figure, panel a). APP is produced in several different isoforms ranging in size from 695 to 770 amino acids. The most abundant form in brain (APP695) is produced mainly by neurons, and differs from longer forms of APP in that it lacks a kunitz-type protease inhibitor sequence in its ectodomain[4,17]. Enzyme activities involved in cleavage of APP at the α-, β- and γ-secretase sites are being identified (see ref. 3 for review). The identity of α-secretase remains unclear, although TACE (an enzyme responsible for cleavage of members of the TNF receptor family at the cell surface) and ADAM9 and ADAM10 are candidates[64,65]. Cleavage of APP by α-secretase releases secreted (s)APPα from the cell surface and leaves an 83-amino-acid carboxy-terminal APP fragment (C83). Production of sAPPα increases in response to electrical activity and activation of muscarinic acetylcholine receptors, suggesting that neuronal activity increases α-secretase cleavage of APP[17]. Amyloidogenic processing of APP involves sequential cleavages by BACE and γ-secretase at the N and C termini of Aβ, respectively. The 99-amino-acid C-terminal fragment of APP generated by BACE cleavage can be internalized and further processed by γ-secretase to produce Aβ40/42 in endocytic compartments. Cleavage of C99 by γ-secretase liberates an APP intracellular domain that can translocate to the nucleus where it may regulate gene expression, including the induction of apoptotic genes[66]. Cleavage of APP/C99 by caspases produces a neurotoxic peptide (C31)[67].

γ-secretase, which cleaves APP within a transmembrane region, involves four different proteins, presenilin, nicastrin, Aph-1 and Pen-2 (Box 1 figure, panel b). The active site of γ-secretase requires the aspartyl protease activity of PS1 conferred by aspartate residues in adjacent transmembrane domains of the C- and N-terminal cleavage fragments of PS1 (red star). Nicastrin, Pen-2 and Aph-1 are each critical components of γ-secretase and each may modify enzyme activity in specific ways and in response to physiological stimuli[68–70]. APP is only one of several proteins that is cleaved by γ-secretase, with Notch-1 being another well-studied γ-secretase substrate[4]. Notch-1 is a cell surface receptor that, when activated by ligands such as Jagged and Delta, is cleaved at the membrane resulting in the release of an intracellular domain of Notch. The intracellular domain of Notch then translocates to the nucleus, where it regulates the transcription of various genes. γ-secretase-mediated Notch-1 signalling plays an essential part in the regulation of cell fate during development of many organ systems including the brain as indicated by embryonic lethality and defective neurogenesis that is identical in Notch-1- and PS1-deficient mice[71].

The normal functions of APP are not fully understood, but increasing evidence suggests it has important roles in regulating neuronal survival, neurite outgrowth, synaptic plasticity and cell adhesion[17]. APP is transported along axons to presynaptic terminals where it accumulates at relatively high levels, which can result in Aβ deposition at synapses[72]. One possible function of full-length APP is as a cell surface receptor that transduces signals within the cell in response to an extracellular ligand[73]. However, neither a ligand nor downstream signalling cascades for APP have been clearly established. Physiological roles for sAPPα are supported by data showing that sAPPα is released from presynaptic terminals in response to electrical activity, and that sAPPα regulates neuronal excitability and enhances synaptic plasticity and learning and memory, possibly by activating a cell surface receptor that modulates the activity of potassium channels and also activates the transcription factor NF-κB[16].

The initial experiments showing that synthetic fragments of Aβ can kill cultured neurons[74] led to a series of studies that have revealed the chemical and cell biological bases for the synaptic dysfunction and death of neurons in AD. Aβ may be most toxic when it is in the form of soluble oligomers in the earliest stages of aggregation[75,76]. Synapses may be particularly susceptible to the adverse effects of aggregating forms of Aβ, as is suggested by the ability of Aβ to impair synaptic ion and glucose transporters and by electrophysiological studies showing that Aβ impairs synaptic plasticity[17,77]. Aβ may damage neurons by inducing oxidative stress and disrupting cellular calcium homeostasis[17]. Coincident with the increased production of Aβ in AD is a decrease in the amount of sAPPα produced, which may contribute to the demise of neurons because sAPPα is known to increase the resistance of neurons to oxidative and metabolic insults[17]. In APP mutant mice memory deficits become apparent relatively early in the process of Aβ deposition, consistent with the neurotoxic effects of Aβ occurring during the formation of oligomers of the peptide[78]. Adding to the evidence that Aβ deposition is a pivotal event in AD is the remarkable finding that immunization of APP mutant mice with human Aβ42 results in the removal of Aβ deposits from the brain[44], which may result in reversal of cognitive deficits[18].



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in several different pedigrees, and the presenilin-1 (PS1) gene was identified as the affected gene[5]. Mutations in a gene on chromosome 1 with high homology to PS1, now called presenilin-2 (PS2), were then shown to cause a few cases of inherited AD[6].

The vast majority of cases of AD are sporadic—they do not run in families. Nevertheless, molecular genetic analyses suggest that there are likely to be many genes that influence one's susceptibility to AD. The first such susceptibility gene identified was apolipoprotein E for which there are three alleles that encode three different isoforms of apolipoprotein E (E2, E3 and E4). Individuals that produce the E4 isoform are at increased risk of AD[7]. The mechanism whereby E4 promotes AD is not established, but there is evidence that E4 enhances Aβ aggregation and reduces Aβ clearance. In addition, data suggest that E4 might increase the risk of AD by enhancing amyloidogenic processing of APP, promoting cerebrovascular pathology, increasing oxidative stress and impairing neuronal plasticity. A second susceptibility locus for late-onset AD has been localized to chromosome 10, but the gene responsible has not yet been established[8].

Risk factors

As with other age-related diseases (cardiovascular disease, diabetes, cancers, and so on), there are likely to be behavioural, dietary and other environmental factors that may affect the risk of AD. However, this area of research has not yet matured to a point where clear recommendations can be made. Epidemiological findings suggest that a low education level, history of head trauma, consumption of high-calorie, high-fat diets and a sedentary lifestyle may each increase the risk of AD[9,10]. When rodents are maintained in a cognitively stimulating environment or on a dietary restriction regimen, neurons in their hippocampus are more resistant to death and neurogenesis (the production of new neurons from stem cells) is increased[10–12]. Similarly, regular physical exercise enhances hippocampal synaptic plasticity and neurogenesis, and is neuroprotective[13].

Specific dietary components may affect the risk of AD. Individuals with low dietary folate intakes are at increased risk of AD, as an apparent consequence of increased levels of homocysteine; studies of mouse models of AD have demonstrated adverse effects of low dietary folate levels and high homocysteine levels on the disease process[10]. Other dietary factors implicated as risk factors for AD include lipids and metals such as copper and iron[9,14]. However, despite accumulating data suggesting that dietary factors may influence disease risk, a causal relationship between caloric intake, or any specific dietary component, and AD has not been established.

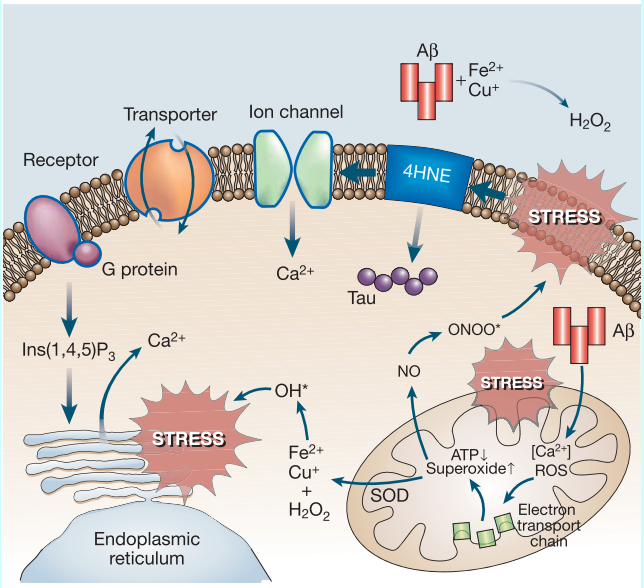

Figure 2 The neurotoxic action of Aβ involves generation of reactive oxygen species and disruption of cellular calcium homeostasis. Interactions of Aβ oligomers and Fe^{2+} or Cu^{+} generates H_2O_2. When Aβ aggregation occurs at the cell membrane, membrane-associated oxidative stress results in lipid peroxidation and the consequent generation of 4-hydroxynonenal (4HNE), a neurotoxic aldehyde that covalently modifies proteins on cysteine, lysine and histidine residues. Some of the proteins oxidatively modified by this Aβ-induced oxidative stress include membrane transporters (ion-motive ATPases, a glucose transporter and a glutamate transporter), receptors, GTP-binding proteins ('G proteins') and ion channels (VDCC, voltage-dependent chloride channel; NMDAR, *N*-methyl-D-aspartate receptor). Oxidative modifications of tau by 4HNE and other reactive oxygen species can promote its aggregation and may thereby induce the formation of neurofibrillary tangles. Aβ can also cause mitochondrial oxidative stress and dysregulation of Ca^{2+} homeostasis, resulting in impairment of the electron transport chain, increased production of superoxide anion radical and decreased production of ATP. Superoxide is converted to H_2O_2 by the activity of superoxide dismutases (SOD) and superoxide can also interact with nitric oxide (NO) via nitric oxide synthase (NOS) to produce peroxynitrite (ONOO*). Interaction of H_2O_2 with Fe^{2+} or Cu^{+} generates the hydroxyl radical (OH*), a highly reactive oxyradical and potent inducer of membrane-associated oxidative stress that contributes to the dysfunction of the ER.

Cleaving APP and unleashing Aβ

A fundamental abnormality that plays a pivotal role in the dysfunction and death of neurons in AD is altered proteolytic processing of APP, resulting in increased production and accumulation in the brain of neurotoxic forms of Aβ. The evidence supporting the "amyloid hypothesis" of AD is extensive and has recently been reviewed[4]. In every case of autosomal dominant early-onset familial AD where the genetic abnormality has been identified (mutations in the APP, PS1 and PS2 genes), the defective gene causes an increase in the production of the long 42-amino-acid form of Aβ (Aβ42) in patients, in cultured cells and in transgenic mice[15,16]. Three different enzyme activities have been identified that determine whether and which form of Aβ is produced (Box 1). The production of Aβ requires sequential cleavages of APP by β-secretase (BACE) at the N terminus of Aβ and by γ-secretase at the C terminus of Aβ. Alternatively, an enzyme called α-secretase cleaves APP within the Aβ sequence, thereby precluding production of Aβ. Mutations in APP that cause familial AD result in one or two amino-acid changes within or immediately adjacent to Aβ that enhance its cleavage by BACE and γ-secretase, whereas presenilin mutations alter γ-secretase activity[4]. The causes of altered APP metabolism and Aβ deposition in sporadic cases of AD are not understood, but may include age-related increases in oxidative stress, impaired energy metabolism and perturbed cellular ion homeostasis.

In addition to the histopathological and genetic evidence supporting a central role of APP mismetabolism in AD pathogenesis, experimental findings in cell culture and animal models have identified adverse consequences of altered APP metabolism consistent with its involvement in synaptic dysfunction and nerve cell death in AD (Box 1). The increased Aβ deposition that occurs in AD most probably contributes to the demise of neurons because Aβ can be directly toxic to neurons and also greatly increases their vulnerability to oxidative and metabolic stress, and excitotoxicity[17]. The ability of immunization-based therapies that remove Aβ from the brains of APP mutant mice to improve synaptic function provides further evidence that Aβ plays a pivotal role in AD and also suggests that the process might be reversible (refs 4 and 18; see also section 'Pathways away from AD' below).

Renegade radicals and power cuts

Increased oxidative stress (uncontrolled production of highly reactive oxygen radicals) and impaired cellular energy metabolism are features of many prominent age-related diseases, and AD is no

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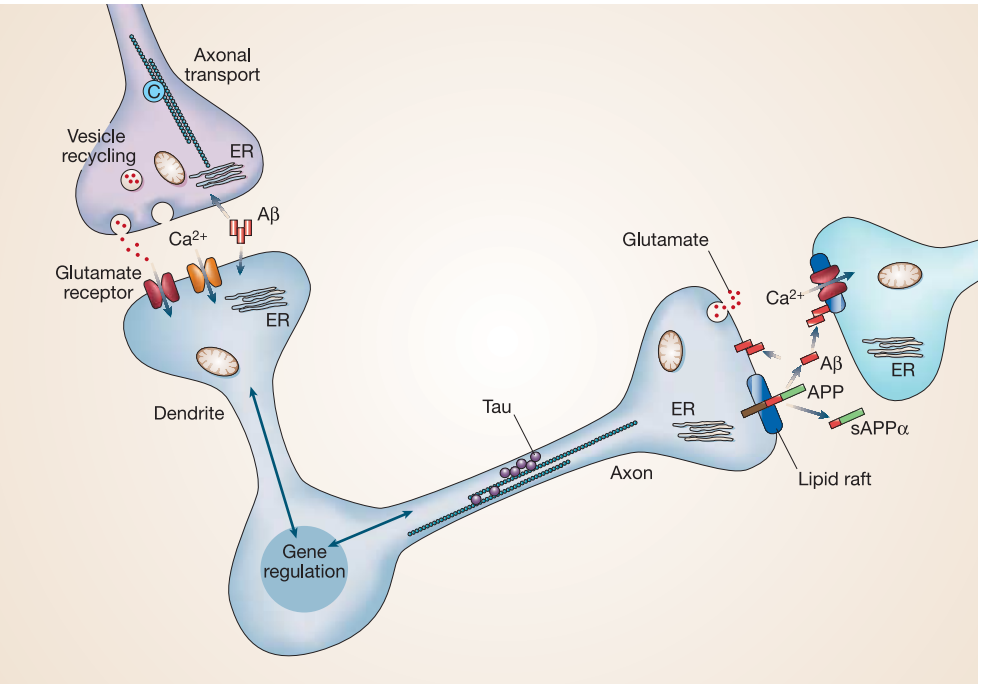
Box 2
The weakest link?

Elaborate neuronal circuits with intricate morphologies and multiple synaptic communication sites allow the brain rapidly to process and store large amounts of information. Axons and dendrites that extend long distances from the cell body are exposed to many different environmental conditions and signalling molecules. To survive and function properly, synapses and axons possess local homeostatic mechanisms that protect them against adverse conditions, including those associated with ageing and disease. Several factors may render synapses and axons vulnerable in AD, including their high content of the disease-related proteins APP, presenilins and tau (axons), and their high metabolic and oxidative loads (Box 2 figure). Synaptic activity regulates APP processing and Aβ released from synaptic terminals may, in turn, modify synaptic plasticity[79]. Because APP is axonally transported and processed in presynaptic terminals, synapses are sites where oligomers of Aβ may accumulate in high amounts[80]. Soluble oligomers of Aβ42 inhibit long-term potentiation (a memory-related form of synaptic plasticity) in the hippocampus of rodents, and increasing evidence suggests that such oligomers are responsible for memory impairment in AD patients[81]. In addition to Aβ, increased production of C-terminal fragments of APP may promote synaptic dysfunction and degeneration in AD[82]. In transgenic mouse models of AD, synaptic dysfunction and memory impairment can occur in the absence of any overt evidence of amyloid deposition or neuronal degeneration[81,83,84]. Synapses are likely to be the sites at which neuronal death is initiated in AD because they contain most of the biochemical machinery for the initiation and execution of apoptosis, and Aβ can induce apoptotic cascades in synapses (ref. 85 and see Box 3).

Many of the neurons affected in AD are relatively large and have long axons. Analyses of AD patients suggest that damage to axons of such neurons may occur relatively early in the disease process[86]. Alterations in axonal transport have been suggested to play a role in AD. APP is axonally transported to synaptic terminals in brain regions affected in AD, suggesting a role for axonal transport in plaque biogenesis[87]. Other findings suggest that abnormalities in tau may contribute to axonal transport deficits in AD[88], consistent with tau's function as an axonal protein that regulates microtubule stability. Perhaps the most compelling evidence that tau and axonal microtubule abnormalities play a fundamental role in the neurodegenerative process in AD comes from the causal role for tau mutations in familial frontotemporal lobe dementia with Parkinsonism, linked to chromosome 17 and elucidation of the pathogenic actions of these tau mutations in cell culture and animal models[89]. Finally, alterations in the amounts and localization of synaptic proteins in vulnerable brain regions in AD patients have been documented[90], and may be involved in the early dysfunction of synapses. In particular, evidence is emerging that the process of synaptic vesicle recycling may be abnormal in AD[91]. Several proteins that regulate clathrin-mediated vesicle recycling are reduced in AD including synaptotagmin, dynamin, AP2 and AP180. Impairment of vesicle recycling might cause the enlargement of axon terminals documented in ultrastructural studies of AD and might also result in impaired neurotrophic factor signalling which involves clathrin-mediated endocytosis of activated receptors.

Perturbed processing of APP resulting in increased production of Aβ at synapses may be an early event in AD (Box 2 figure). APP is axonally transported and Aβ therefore likely accumulates at synapses in high amounts in AD. Aβ can have multiple adverse effects on the functions and integrity of both pre- and postsynaptic terminals including inducing oxidative stress, impairing calcium homeostasis and perturbing the functions of mitochondria and the ER. Abnormalities in axons may result from adverse effects of Aβ on tau, and microtubules resulting in neurofibrillary tangle formation and cell death. Presynaptic disturbances in synaptic vesicle trafficking and axonal transport may also contribute to the dysfunction and death of neurons in AD. Oxidative stress, perturbed calcium regulation and mitochondrial impairment are major alterations involved in functional and structural abnormalities in synapses and axons in AD.



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exception. Cells in the brains of AD patients exhibit abnormally high amounts of oxidatively modified proteins, lipids and DNA; such free-radical-mediated molecular damage is particularly prominent in the environment of plaques and in neurofibrillary tangle-bearing neurons, suggesting roles for oxidative stress in amyloid-mediated neuronal damage and neurofibrillary pathologies[19]. Several sources of oxidative stress in AD have been proposed, with Aβ and redox-active metals such as Fe^{2+} and Cu^{+} being two such sources[14,19,20]. During the process of aggregation Aβ generates hydrogen peroxide, a process that requires oxygen and that is greatly potentiated by Fe^{2+} and Cu^{+} (refs 13, 18). Lipid peroxidation induced by Aβ impairs the function of ion-motive ATPases, glucose and glutamate transporters, and also GTP-binding proteins as the result of covalent modification of the proteins by the aldehyde 4-hydroxynonenal[17]. By disturbing cellular ion homeostasis and energy metabolism, relatively low levels of membrane-associated oxidative stress can render neurons vulnerable to excitotoxicity and apoptosis. The dysfunction and degeneration of synapses in AD may involve Aβ-induced oxidative stress, because exposure of synapses to Aβ impairs the function of membrane ion and glutamate transporters and compromises mitochondrial function by an oxidative-stress-mediated mechanism (Fig. 2).

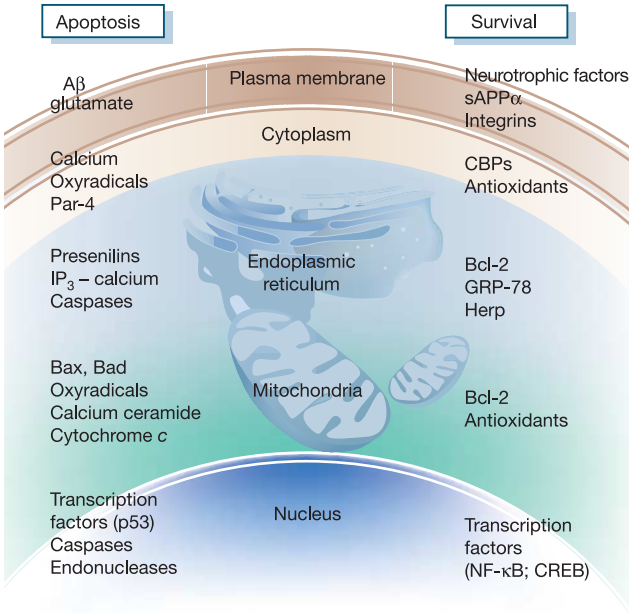
Brain imaging studies have demonstrated deficits in glucose use in living AD patients, an abnormality that may occur before the onset of clinical symptoms[21]. The activities of cytochrome *c* oxidase, pyruvate dehydrogenase complex and α-ketoglutarate dehydrogenase complex—critical enzymes in energy metabolism—are decreased in brain cells of AD patients[21]. Altered proteolytic processing of APP may contribute to impaired energy metabolism, because brain glucose metabolism is decreased in cognition-related brain regions of APP mutant mice in association with increased amounts of Aβ in their brains[13,22]. Moreover, hypoxic tolerance is significantly

Box 3
The death of neurons

Apoptosis is a form of programmed cell death that involves changes in the cytoplasm, ER, mitochondria and nucleus (Box 3 figure). It typically includes the production and/or activation of proteins such as Bax and Bad that increase the permeability of mitochondrial and ER membranes resulting in the release into the cytoplasm of cytochrome *c* from the mitochondria and calcium from the ER[92]. The latter events then activate enzymes called caspases that cleave various protein substrates to sculpt morphological and biochemical aspects of the cell death process. For example, cleavage of cytoskeletal proteins and ion channel proteins causes cell shrinkage and may prevent necrosis, whereas cleavage of DNA degrades chromosomes. Evidence that many neurons undergo apoptosis in AD includes the presence of high levels of activated apoptotic proteins such as caspase-3 and Bax in neurons that exhibit neurofibrillary tangle pathology[93,94]. In addition, DNA damage and upregulation of the pro-apoptotic proteins p53 and Bax occur in vulnerable neuronal populations at a relatively early stage in the disease process. Familial AD (FAD) mutations in presenilins render neurons vulnerable to apoptosis induced by Aβ, trophic factor deprivation and other stimuli[93], consistent with an apoptotic mode of neuronal death in patients with these presenilin mutations. APP mutations are also sufficient to trigger apoptosis in cultured cells[95]. AIF, apoptosis-inducing factor; ATM, ataxia telangiectasia mutated; Apaf-1, apoptotic protease-activating factor 1. CREB, cyclic AMP binding protein; CBP, CREB binding protein; IP_3, inositol 1,4,5 trisphosphate

The triggers of cell death in AD may include Aβ, activation of glutamate receptors, increased oxidative stress, DNA damage and elevation of intracellular calcium levels. Once triggered, apoptosis proceeds with the production and/or activation of proteins such as p53, Bax, Bad and Par-4 that induce mitochondrial membrane permeability changes. Release of cytochrome *c* and AIF (apoptosis-inducing factor) from mitochondria and release of calcium from the ER occurs, followed by activation of the apoptosome, a protein complex that includes cytochrome *c*, Apaf-1 and caspases 9 and 3. Finally, cleavage of various substrate proteins by caspases and endonucleases occurs. Neurotoxic forms of Aβ may be a trigger of apoptosis in AD because pro-apoptotic proteins are associated with Aβ deposits in the brains of AD patients and Aβ can induce apoptosis of cultured neurons[93,94]. Exposure of neurons to Aβ induces an apoptotic cascade that involves: upregulation of p53, Bax and Par-4; mitochondrial membrane permeability transition and release of cytochrome *c*; activation of the apoptosome resulting in caspase-3 activation; and nuclear chromatin fragmentation. Neurons treated with inhibitors of p53, agents that stabilize mitochondrial and ER membranes or caspase inhibitors are resistant to being killed by Aβ[93,94]. In addition to Aβ, C-terminal proteolytic products of APP have been implicated in neuronal apoptosis[67,95].

In addition to mitochondrial alterations, abnormalities in the ER and nucleus have been documented in studies of AD patient tissue and cell culture and animal models. FAD PS1 mutations have been shown to render neurons vulnerable to apoptosis, apparently by altering ER stress responses and ER calcium regulation[29,84]. Apoptosis in AD may involve DNA-damage-response pathways and inappropriate activation of cell cycle pathways involving the cyclin-dependent kinase 5 and its neuron-specific activator p35. As evidence, proteolytic cleavage of p35 produces p25, which accumulates in the brains of AD patients and in cultured neurons exposed to Aβ1-42 (ref. 96), and Aβ-induced neuronal death is mediated by the ATM-dependent DNA-damage-response pathway[97]. Increasing evidence suggests that ubiquitin-proteasomal degradation of proteins is impaired in AD, resulting in the abnormal accumulation of damaged proteins in neurons (UBB + 1 mutations)[98]. In this regard it was recently reported that a ubiquitin-conjugating enzyme called E2-25K/Hip-2 mediates Aβ-induced inihibition of proteasome activity, which is required for Aβ-induced apoptosis[99]. There are several mechanisms that can protect neurons against apoptosis, including activation of cell surface receptors coupled to anti-apoptotic pathways, such as those activated by neurotrophic factors and sAPPα[16], and agonists that activate the cyclic AMP second messenger pathway[17,100].



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decreased in presymptomatic APP mutant mice, suggesting an early role for perturbed energy metabolism in the pathogenic action of altered APP processing[23]. There is increasing evidence that a systemic abnormality in glucose regulation, and insulin resistance in particular, is a risk factor for AD[24], although the possible link between diabetes and AD requires further investigation. The ability of dietary restriction, which enhances insulin sensitivity, to protect neurons in experimental models relevant to AD supports a role for perturbed glucose metabolism in AD pathogenesis[10], although this remains to be established in humans. Because oxidative stress and impaired energy metabolism can induce amyloidogenic processing of APP, resulting in accumulation of potentially neurotoxic forms of Aβ, it has been suggested that such cellular stresses may promote amyloidogenesis[25]. The latter mechanism could contribute to increased amyloid production in late-onset forms of AD, because oxidative stress and metabolic impairment increase with advancing age.

Uncontrollable calcium

The calcium ion (Ca^{2+}) plays fundamental roles in learning and memory and is also involved in neuron survival and death. The inability of neurons to regulate calcium homeostasis is an aspect of AD pathogenesis that appears to be intimately involved in the dysfunction and death of neurons[25]. Studies of AD patients and of the pathogenic actions of APP and PS1 mutations support a role for perturbed calcium regulation in AD. For example, neurofibrillary tangle-bearing neurons exhibit high amounts of Ca^{2+} and evidence of hyperactivation of Ca^{2+}-dependent proteases and Ca^{2+}-activated kinases[26]. AD-causing mutations in PS1 and APP perturb cellular calcium homeostasis in cultured neurons and transgenic mice. Perturbed processing of APP may destabilize calcium homeostasis in neurons by increasing production of Aβ42 and by decreasing levels of sAPPα[25]. Aβ may perturb calcium regulation by inducing oxidative stress, which impairs membrane calcium pumps and enhances calcium influx through voltage-dependent channels and ionotropic glutamate receptors (Fig. 2). Other findings suggest that Aβ can promote Ca^{2+} influx by forming channels in membranes or by activating cell surface receptors coupled to calcium influx[27,28].

Presenilin mutations and amyloidogenic processing of APP can destabilize Ca^{2+} homeostasis in astrocytes, oligodendrocytes and microglia, which might contribute to white-matter damage and local inflammatory processes[27]. AD-causing PS1 mutations have been shown to alter Ca^{2+} regulation by increasing the amount of Ca^{2+} that can be released from the endoplasmic reticulum (ER) in neurons stimulated by glutamate, membrane depolarization or agonists that activate receptors coupled to inositol-1,4,5-trisphosphate (Ins(1,4,5)P_3) production[29]. Moreover, a defect in capacitative calcium entry has been documented in cells expressing mutant PS1. However, presenilin mutations are a very rare cause of AD, and there is no evidence that a Ca^{2+}-regulating action of PS1 is involved in sporadic forms of AD. Disturbances of Ca^{2+} regulation in AD may not be limited to neurons. Nevertheless, recent findings from studies of neurons lacking PS1 suggest that wild-type PS1 may normally serve a Ca^{2+}-regulating function in the ER[30]. Interestingly, studies of lymphocytes from patients with familial or sporadic AD, and from APP and PS1 mutant mice, have demonstrated abnormalities in calcium signalling reminiscent of those in neurons[31]. Thus, perturbed cellular Ca^{2+} homeostasis appears to be a widespread abnormality in both familial and sporadic forms of AD that may contribute to the disease process.

Feeding in the fats

Lipids are structural components of cell membranes, serve as intra- and intercellular signalling molecules and can modify the functions of many different proteins. Individuals that consume diets high in cholesterol and those with increased cholesterol levels may be at increased risk of AD, whereas those who take cholesterol-lowering drugs (statins) may be at reduced risk[32,33]. The well-known adverse effects of high-cholesterol diets on the vasculature could contribute an increased risk of AD. However, accumulating data suggest that cholesterol may feed more directly into the amyloid cascade by promoting amyloidogenic processing of APP. Statin treatment decreases Aβ levels and plaque formation in APP mutant transgenic mice[34], and high levels of cholesterol and shifts in subcellular cholesterol metabolism can increase the production of Aβ in cultured cells and APP mutant transgenic mice[32]. Alterations in

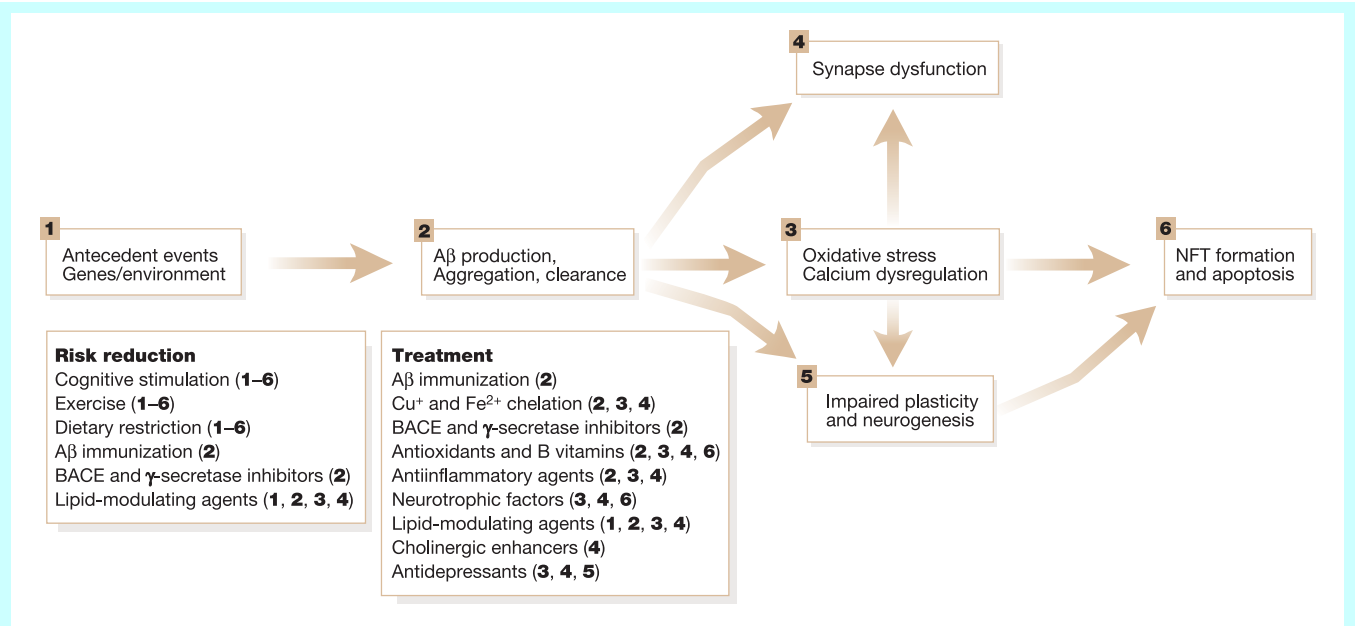

Figure 3 Strategies and targets for the prevention and treatment of AD. Approaches that are being tested in clinical and/or primary prevention trials include Aβ immunization, Cu^{+}/Fe^{2+} chelation, cholesterol-lowering drugs (statins), anti-inflammatory agents, antioxidants and folic acid. Epidemiological and animal studies suggest the potential benefits of cognitive stimulation, regular physical exercise and dietary restriction.

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cholesterol metabolism might also promote neuronal degeneration by perturbing membrane fluidity and signal transduction. Increasing evidence suggests that diets high in saturated fats may increase the risk of AD, whereas diets rich in mono- and polyunsaturated fatty acids and omega-3 fatty acids may decrease disease risk, and several studies suggest that diets rich in omega-3 fatty acids, such as those found in fish, can reduce the risk of AD[35]. Although the emerging data linking cholesterol and fatty acids to AD are encouraging, the potential of dietary modifications of fat intake to affect disease risk remains to be established.

Microdomains of plasma membranes called lipid rafts, in which the outer leaflet of the lipid bilayer is enriched in cholesterol and sphingomyelin may be sites at which several molecular events implicated in AD pathogenesis occur, including synaptic signal transduction, APP processing and the initiation of apoptosis[36]. Analyses of raft lipids in brain tissue samples from AD and control subjects have demonstrated increased amounts of cholesterol and ceramides in AD, which are associated with increased levels of membrane-associated oxidative stress[37]. Exposure of neurons to Aβ or other oxidative insults results in increased accumulation of cholesterol and ceramides in the neurons and these alterations can be prevented by treating the neurons with vitamin E and inhibitors of ceramide[37], suggesting that increased membrane oxidative stress may induce the production of cytotoxic ceramides in AD. Further studies in animal models and AD patients are required to clarify the roles of perturbed lipid metabolism in AD.

Synaptic and axonal alterations short circuit cognition

Learning and memory requires information exchange between neurons in circuits that pair at least two different environmental inputs in space and time. For example, when we meet someone new visual and auditory pathways are activated and this information is routed to the hippocampus, a brain region in which the convergent activity leads to enduring changes in synaptic strength so that the next time we see the person or hear his or her voice we remember his or her name. For reasons that remain elusive, neuronal circuits critical for learning and memory are particularly vulnerable to dysfunction and degeneration in AD. Emerging evidence implicates subtle changes in the function and structure of synapses and axons in these brain regions as being early and pivotal events in the pathogenesis of the neurodegenerative process in AD (Box 2). Consistent with this possibility, drugs that enhance activation of those synapses, such as acetylcholinesterase inhibitors, can improve cognition during the early stages of clinical disease[38].

A neuron's demise

Loss of neurons in the entorhinal cortex, hippocampus, frontal, parietal and temporal cortices of AD patients has been documented[39]. Neurons in layer II of the entorhinal cortex and hippocampal CA1 neurons are particularly vulnerable. The reason(s) for this selective vulnerability remains uncertain, but might be related to the expression of genes that either promote or prevent neuronal death, including glutamate receptors, calcium-binding proteins and neurotrophic factors. The pattern of neuronal loss in AD overlaps with, but is not identical to, that of normal ageing—suggesting that AD pathogenesis is not simply an acceleration of normal brain ageing. Large numbers of neurofibrillary tangle-bearing neurons occur in AD, but neuronal loss exceeds the number of neurofibrillary tangles, which suggests that tangle-bearing neurons are removed and/or that some neurons die without forming tangles[40].

The death of populations of neurons in the brain regions affected in AD apparently occurs over a prolonged time period of many years, which suggests that a relatively small number of neurons are dying at any one time. Such a spatio-temporal pattern of cell death is characteristic of a form of programmed cell death called apoptosis and contrasts with necrosis in which cells die *en masse*. Increasing evidence suggests that many neurons may die by apoptosis in AD (Box 3 and ref. 41), although it may not be the only form of cell death.

Of glia and microglia

Glial cells (astrocytes, oligodendrocytes and microglia) greatly outnumber neurons in the brain; they express many of the genes linked to AD and are subjected to many of the same environmental conditions as are neurons. Alterations in each of the three types of glial cells have been documented in studies of AD patients and of animal and cell culture models of AD, and available data suggest that Aβ plays a role in inducing many of the alterations in glial cells[42]. Abnormalities in astrocytes that may contribute to synaptic dysfunction and neuronal death include impaired glutamate transport, perturbed calcium regulation and production of pro-inflammatory cytokines. White matter consists of axons and oligodendrocytes, the cells that wrap around the axons, insulating them, and thereby facilitate rapid conduction of action potentials. Degeneration of cells in white matter is prominent in the brains of AD patients, and oligodendrocytes are vulnerable to being damaged and killed by Aβ[43].

Microglia are macrophage-like cells that play important roles in responses of the brain to injury and infection. In AD, activated microglia congregate around amyloid plaques and degenerating neurons, and may produce toxins and inflammatory cytokines that contribute to the neurodegenerative process[42]. Both innate and cell-mediated immune mechanisms are involved in the pathogenesis of AD. In addition to activation of microglia, the innate immune response includes engagement of the classical complement cascade and induction of chemokines and pentraxins[42]. Evidence for participation of cell- and antibody-mediated responses in AD pathogenesis comes from recent studies demonstrating the ability of the immune system to generate antibodies against Aβ that may promote removal of Aβ from the brain[44,45].

Whereas many of the changes in glial cells in AD may promote neuronal degeneration, some of the changes may represent adaptive responses aimed at promoting neuronal plasticity and survival. For example, microglia may also remove Aβ, a potentially beneficial action of these immune cells[46]. In addition, although synapses degenerate in vulnerable neuronal circuits, the remaining synapses may increase in size to compensate and astrocytes may play a role in this process[47,48]. Moreover, the production of neurotrophic factors such as basic fibroblast growth may increase in astrocytes associated with Aβ deposits[49]—such neurotrophic factors as well as certain cytokines[50] may counteract the neurodegenerative process in AD.

Pathways away from Alzheimer's disease

Diet and lifestyle

Potentially effective means of decreasing disease risk, and of inhibiting or even reversing the AD disease process in symptomatic patients, are emerging from recent studies (Fig. 3). Although not yet fully established, available data suggest that cognitively stimulating environments, physical exercise and diets low in calories and 'bad' fats (cholesterol and saturated fats) may reduce the risk of AD[9,10,51]. The latter epidemiological findings are supported by animal studies showing that cognitively stimulating environments, physical exercise and dietary restriction regimens increase the resistance of neurons in the brain to degeneration, enhance neurogenesis and improve learning and memory[10–12]. Exercise, cognitive stimulation and dietary restriction may each exert a beneficial effect through a similar mechanism involving increased production of brain-derived neurotrophic factor (BDNF)[10–12], although the importance of BDNF in AD remains to be determined. Vitamin supplementation may reduce disease risk, as suggested by a recent prospective study demonstrating reduced prevalence and incidence of AD in individuals taking vitamins C and E[52], and the association of high folic acid levels and decreased homocysteine levels with reduced disease risk[10,53]. The possibility that one's risk for AD can be reduced by

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modifications of diet and lifestyle is of considerable interest, and suggests the potential for reducing the incidence of AD by preventative strategies similar to those that reduce the risk of cardiovascular disease.

Targeted therapeutics

Drugs are in development that target specific sites in the neurodegenerative cascade. Because the production of neurotoxic forms of Aβ from APP appears to be a pivotal event in AD pathogenesis, there is intense interest in developing drugs that block the β- or γ-secretase enzymes. Specific γ-secretase inhibitors that decrease Aβ production have been produced[54], but their use in humans may be compromised by side effects resulting from blockade of γ-secretase cleavage of Notch and other protein substrates. BACE inhibitors[55] may prove beneficial in reducing Aβ production without major side effects, because BACE-deficient mice have reduced Aβ production and do not exhibit any overt abnormal phenotypes[56]. Another approach to reducing amyloid accumulation in the brain is agents that chelate copper and iron[14,57]; such chelators would also be expected to reduce oxidative stress in neurons. The emerging link between cholesterol levels and AD has led to trials of cholesterol-lowering statins in AD patients (http://www.alzforum.org/dis/tre/drc).

One promising approach for preventing and treating AD is based upon stimulating the immune system to remove Aβ from the brain. The initial reports that immunization with human Aβ42 (ref. 58) or passive immunization with Aβ antibodies[59] result in the clearance of Aβ plaques from the brains of APP mutant transgenic mice were followed by reports that such immunization approaches can also ameliorate memory deficits in the mice[18,60]. The results of an initial clinical trial suggest that Aβ immunization may be an effective treatment for AD, although adverse reactions occurred in some patients and refinement of the immunization methods will be required[61]. In addition, the rate of cognitive decline in the AD group that received the vaccine but did not respond by plaque clearance was much greater than is typically seen in AD patients. Therefore, although the vaccine responders were cognitively better than the non-responders, their rate of cognitive decline was similar to that typical for patients with sporadic AD. It should also be noted that there have been many failures of trials in human patients of treatments that worked well in animal models of other neurodegenerative disorders, including Parkinson's disease and stroke. Nevertheless, an effective vaccine would have a major impact on the disease, and is currently one of the most exciting areas of AD research.

Other therapeutic approaches being tested include anti-inflammatory agents such as COX-2 inhibitors and aspirin[62], and steroids that decline during normal ageing such as oestrogen and testosterone[63]. Drugs that target specific sites in neurodegenerative cascades have, to date, only been employed in cell culture and animal models of AD, and their potential in the clinic remains unclear. Finally, the ability to identify individuals at risk for AD, based upon genetic (Apo E4 genotype, for example) or environmental (overweight sedentary lifestyle) factors, will allow the application of more aggressive interventions in those individuals. □

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