Apolipoprotein E and cholesterol metabolism in the pathogenesis and treatment of Alzheimer's disease

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There is much evidence suggesting that there is a strong relationship between the deterioration of brain lipid homeostasis, vascular changes and the pathogenesis of Alzheimer's disease (AD). These associations include: (1) recognition that a key cholesterol transporter, apolipoprotein E type 4, acts a major genetic risk factor for both familial and sporadic AD; (2) epidemiological studies linking cardiovascular risk factors, such as hypertension and high plasma cholesterol, to dementia; (3) the discovery that small strokes can precipitate clinical dementia in cognitively normal elderly subjects; (4) the modulation of degradation of the amyloid precursor protein by cholesterol administration in cell culture and in animal models of β-amyloid overproduction; and (5) the beneficial effect of cholesterol-lowering drugs, such as Probucol and statins, in combating common AD. The recent finding that there is a genetic association between the *HMGR* gene locus and sporadic AD further suggests that brain cholesterol metabolism is central to AD pathophysiology, and a potential therapeutic target for disease stabilization and primary disease prevention.

Alzheimer's disease (AD) is a multifactorial disease with a strong genetic component, and can be subdivided into two distinct types, the 'familial' and 'sporadic' forms. AD is associated with neuronal loss, synaptic damage, deposition of β-amyloid, accumulation of neurofibrillary tangles and loss of cholinergic activity in susceptible brain regions. The discovery of genetic linkages and the identification of genes responsible for AD has greatly enhanced our understanding of the disease. Furthermore, the identification of specific mutations in genes that have been linked with the disease has altered how we perceive the molecular changes responsible for the pathophysiological process that characterizes AD. Three different gene products have so far been shown to cause early-onset familial AD: the amyloid precursor protein (APP), presenilin 1 (PS-1) and presenilin 2 [1]. However, sporadic AD, which represents 85–90% of all cases worldwide, is generally of late onset, occurring after 65 years of age. Although several genetic risk factors (as opposed to causes) have been associated with this form of the disease, only the gene encoding apolipoprotein E type 4 (*apoE4*) has been repeatedly linked with sporadic AD [2,3]; all other putative genetic risk factors have shown poor or controversial replications.

Cardiovascular contribution to AD pathology

The incidence of degenerative and vascular dementias increases almost exponentially with age, from 70 years onwards. Hence, as a result of increasing human longevity, both types of dementia have become major public-health problems worldwide. The integrity of the cerebral vasculature is crucial for the maintenance of cognitive functions, and evidence suggests that cerebrovascular function declines during normal ageing, with pronounced effects evident in Alzheimer's disease. The causes of these changes remain largely unknown. Many studies have recorded age-related impairments, such as atherosclerosis and loss of innervation, in basal surface arteries of the brain, but it has only recently been noted that subtle alterations are also apparent in the intracranial resistance vessels and in the smaller capillaries of ageing animals and humans [4]. The abnormalities observed include profound irregularities in the course of microvessels, unexplained inclusions in the basement membrane, and changes in unique proteins and membrane lipids associated with the blood–brain barrier. Brain imaging and permeability studies have provided no clear functional evidence for these structural and biochemical anomalies, but it is plausible that focal and transient breaches of the blood–brain barrier occur during ageing and, particularly, in the early stages of AD. For example, clinically normal *apoE4* carriers aged over 50 are more likely to show significant cognitive impairment and lower cerebral glucose metabolism by positron emission tomography [5,6]. Even relatively young carriers of *apoE4* (31 ± 5 years) show reduced cerebral glucose metabolism, despite a complete absence of clinical impairments. Interestingly, the *apoE4* allele has been shown to impact significantly on the age of onset of both the familial and sporadic forms of AD [2,3]. These findings suggest that *apoE4* carriers, which are genetically 'at risk' of developing AD, are developmentally different from their non-carrier counterparts. The possibility that hypertension or decreased cerebral blood flow might be major risk factors for sporadic AD has become the target of growing interest.

Epidemiological and neuropathological studies have suggested that there is an association between common

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AD and several vascular risk factors, such as hypertension, inheritance of the allele encoding apolipoprotein-E-ϵ4, myocardial infarctions, diabetes mellitus, ischemic white-matter lesions, generalized atherosclerosis [7] and, most recently, high consumption of animal fat [8]. These findings could reflect an over-diagnosis of AD in individuals with silent cerebrovascular disease, or an effect of cerebrovascular disease on the clinical expression and onset of AD. Alternatively, AD might increase the risk of vascular disease, or vascular conditions might silently stimulate the development of AD. It is possible that similar mechanisms, such as disturbances in lipid homeostatic processes or abnormal cholesterol transport and distribution, are involved in the pathogenesis of both types of disorder.

Several longitudinal studies have suggested that hypertension predisposes individuals to the development of cognitive impairment and ensuing dementia, after a period varying from a few years to several decades. The presence of the *apoE4* allele greatly increases this risk in hypertensive subjects [9]. Anti-hypertensive drug treatments have been shown to reduce the risk of developing cognitive impairment and, in some studies, of developing AD. Estrogen, which is known to cause a reduction in serum cholesterol levels and in the risk of coronary heart disease, also lessened the incidence of dementia in cross-sectional epidemiological studies, but not its progression in double-blind placebo-controlled clinical trials [10].

Cholesterol synthesis, transport and degradation in AD pathophysiology

Over the past 20 years, many different research groups have documented the cholesterol levels in different areas of the brain in AD patients. It is generally agreed that brain cholesterol levels (total, free and esterified) are significantly reduced in cortical and hippocampal areas, but not in the cerebellum, in AD [11–14]. Similar reductions have also been reported for the phospholipid content and for other key lipid contributors to membrane structure. Interestingly, the plasma membrane cholesterol: phospholipid ratio is markedly decreased in the temporal and frontal cortices of AD subjects [15], correlating with a pronounced thinning of plasma membranes in these areas. These pathophysiological findings augment clinical and epidemiological evidence suggesting that elderly individuals with elevated plasma cholesterol have an increased susceptibility to dementia and AD compared with normocholesterolemic subjects [16–18].

Cholesterol homeostasis is carefully maintained in the brain through a series of interdependent processes, including synthesis, storage, degradation and transport. Cholesterol and other lipids are used for membrane synthesis and for many other purposes in cells throughout the body, including those of the central nervous system (CNS), a site of high lipid turnover. Cells of the nervous tissue are capable of *de novo* synthesis of lipid molecules, and can also bind and take up lipoproteins from their local environment. Brain cells cultured *in vitro*, particularly astrocytes and neurons, synthesize cholesterol at a rate that is inversely proportional to the cholesterol content in the growth environment.

The endogenous synthesis of cholesterol involves more than 20 reactions and is regulated primarily by the activity of the 3-hydroxy-3-methylglutaryl-coenzyme-A reductase (HMGR), which catalyzes the formation of mevalonate, the key precursor molecule in the synthesis of cholesterol (Fig. 1). Analysis of HMGR activity in the brains of autopsy-confirmed AD cases revealed a marked reduction in the activity of this enzyme in cortical and hippocampal areas, when compared with age-matched control subjects [13]. This reduction in HMGR enzymatic activity was found to be *apoE*-genotype independent. The *HMGR* gene locus is only a few centimorgans away from a polymorphic DNA marker on chromosome 5 that is associated with late-onset familial AD [19]. Although no difference was observed between HMGR mRNA levels in AD versus control subjects [13,20], an abnormal transcript containing intron M of the gene was detected in nearly all the AD subjects examined [13]. A loss of HMGR activity would certainly be consistent with the reduced cholesterol levels in brain regions affected by AD pathology [11–14].

The uptake of exogenous lipoprotein-derived cholesterol in the brain requires internalization of the lipoprotein (usually an apoE-rich lipoprotein complex) bound to its surface receptor. In the peripheral nervous system it has been shown that apoE can coordinate the mobilization and redistribution of cholesterol for repair, growth and maintenance of myelin and neuronal membranes during development or after injury. In the CNS, apoE plays a pivotal role in cholesterol delivery during the membrane remodeling that is associated with synaptic turnover and dentritic reorganization. The near complete absence in the brain of other key plasma apolipoproteins, such as apoA1 and apoB, further emphasizes the crucial and unique role of apoE in cholesterol transport in the normal and injured CNS. However, we cannot rule out the possibility that apolipoproteins originating from the periphery (such as apoA1 and apoB) could play an active role in the local pathological process during leakage across the blood–brain barrier in areas of severe neurodegeneration.

Ultrastructural studies have shown that a loss of neuronal input to the hippocampus causes astrocytes and microglia progressively to engulf both pre-synaptic terminals and pre-terminal axons (Fig. 2a), thereby rapidly clearing the area to allow synaptic replacement. Once metabolized, terminal-derived ovoids generate a large glial store of lipids that are readily available for the synthesis of membrane components required for new synapses and dendrites (Fig. 2a,b) in surviving adjacent neurons. As the intracellular concentration of cholesterol in glial cells rises, cholesterol synthesis is increasingly repressed by inhibition of HMGR [21]. The accumulation in astrocytes of high concentrations of cellular cholesterol induces the synthesis of apoE, which combines with phospholipids and the cholesterol derived from degenerating terminals to produce a functional high-density-lipoprotein (HDL)-like complex (Fig. 2b). The resulting apoE-lipoprotein complex is secreted into the extracellular space where it is recognized by apoE receptors located on the ependymal cells that surround the ventricles of the brain and by specific neuronal target sites within the CNS.

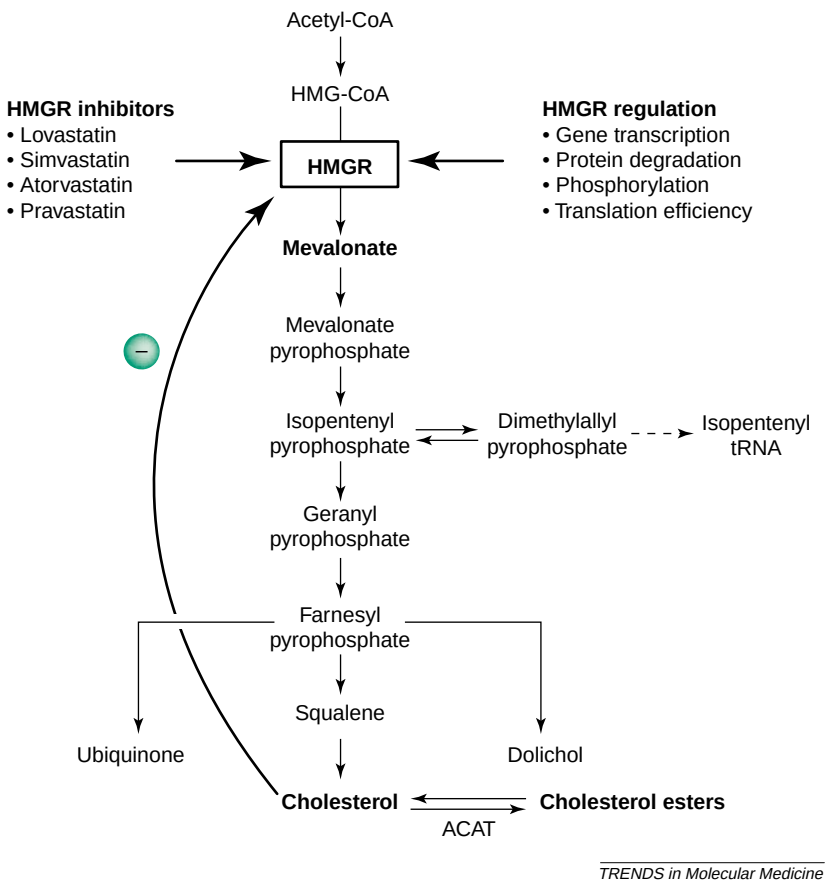

Fig. 1. Cholesterol synthesis in eukaryotic cells: the mevalonate pathway, and key enzymes regulating cholesterol synthesis and esterification. The synthesis of cholesterol is regulated chiefly by the activity of the 3-hydroxy-3-methylglutaryl-coenzyme-A reductase (HMGR), the enzyme that catalyzes the formation of mevalonate from 3-hydroxy-3-methylglutaryl-coenzyme-A (HMG-CoA). Abbreviations: ACAT, acetyl coenzyme A:cholesterol acyltransferase; CoA, coenzyme A.

Deafferented granular cell neurons in the hippocampus have been shown to exhibit markedly increased numbers of low-density lipoprotein (LDL) receptors in the early and middle phases of the re-innervation process (Fig. 2c) [21]. *In vitro*, the so-called apoE–LDL-receptor-related protein (LRP) expressed by embryonic neurons also appears to modulate the internalization of cholesterol-rich lipoproteins, leading to the release of cholesterol in the cell, where it mediates terminal proliferation and axonal extension [22]. Following binding of the apoE complex to a cell-surface apoE receptor, the apoE–cholesterol–LDL-receptor complex is internalized and degraded, releasing cholesterol into the neuron (Fig. 2d).

The released cholesterol is then transported to the dendritic field or to terminals of sprouting or re-innervating neurons for membrane and synapse formation (Fig. 2f). In response to increased internalization of cholesterol via the apoE–LDL-receptor pathway there is a secondary reduction in HMGR activity. Hence, the apparently contradictory notion of depressed cholesterol synthesis during active synaptogenesis can be explained by postulating a mechanism of specific salvage and re-utilization of cholesterol from degenerating terminals through an apoE-transport and LDL-receptor-uptake pathway [23]. In *apoE*-knockout mice, synaptogenesis is compromised, causing cognitive deficits with age. This knockout also prevents amyloid deposition when mice are cross-bred with transgenic APP^{717} overexpressor mice (which are used to model amyloid deposition in the brain). It is noteworthy that secreted β-amyloid is found predominantly bound to apoE lipoproteins in the brain. Neurons and glials cells use LDL-receptor-mediated internalization to scavenge extracellular β-amyloid–lipoprotein complexes and to send them to the endosomal and lysosomal compartments for degradation [24].

Brain cholesterol can be transiently stored in brain cells by conversion of free cholesterol into esterified cholesterol droplets in the cytoplasm, using the 'acetyl coenzyme A: cholesterol acyltransferase' (ACAT) [25]. However, little is known about this process, and the brain does not have sufficient space available for the long-term storage of lipids.

Cholesterol and β-amyloid metabolism: a role for apoE-mediated transport

The first experimental evidence for a strong interplay between cholesterol and β-amyloid metabolism was published by Sparks and colleagues in 1994 [26]. They showed that feeding rabbits a cholesterol-enriched diet for eight

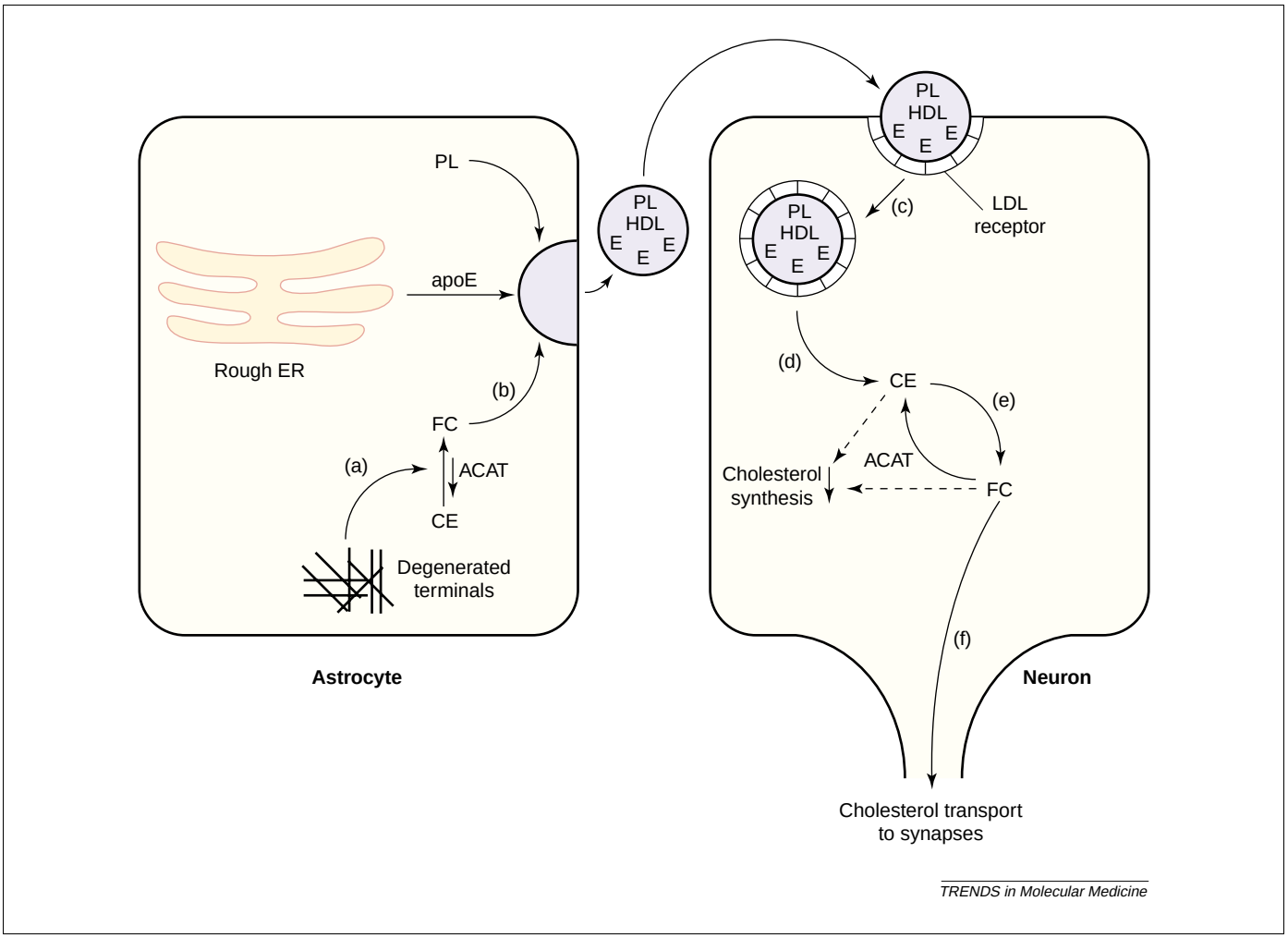

Fig. 2. A hypothesis for the mechanism of cholesterol–phospholipid recycling in the injured central nervous system. Degenerating terminals are internalized by astrocytes and microglia, and degraded to release non-esterified cholesterol (a), which can be used as free cholesterol (FC) for the assembly of apolipoprotein E (apoE)–cholesterol–lipoprotein complexes (b) or converted, by the acetyl coenzyme A:cholesterol acyltransferase (ACAT) into cholesterol esters (CE) for storage purposes. Newly formed apoE–cholesterol–lipoprotein complexes are directed to the circulation (presumably through the ependymal cells that surround the ventricles) and/or to specific brain cells requiring lipids. In the latter case, apoE complexes are thought to be internalized (c) after binding to a neuronal member of the low-density lipoprotein (LDL) receptor family (e.g. the LDL receptor, the very-low-density-lipoprotein receptor, or the apoE–LDL-receptor-related protein). Cholesterol is then released in esterified form (d) and can be converted to FC (e) for use in dendritic proliferation and/or synaptogenesis (f). As a consequence of the internalization process, *de novo* cholesterol synthesis via the mevalonate pathway is repressed. Abbreviations: E, ApoE; HDL, high-density lipoprotein; PL, phospholipid.

weeks led to an accumulation of intracellular β-amyloid immunoreactivity in neurons of the hippocampal area. This observation has since been replicated in African green monkeys receiving a saturated-fat- and cholesterol-rich diet over the course of five years [27]. Transgenic mice expressing the human APP Swedish mutation also show accelerated amyloid deposition in the brain in response to high cholesterol diets [28,29]. Furthermore, cholesterol diet manipulations in humanized-APP-transgenic mice have been used to increase apoE production in the brain and to reduce indirectly the steady-state concentrations of β-amyloid 1–40 and 1–42 in the hippocampal and cortical areas [30]. Previously, Poirier and colleagues [31,32] had reported that functional reconstituted apoE-lipoprotein complexes effectively scavenge extracellular β-amyloid in both primary-astroglial and neuronal cell cultures. This process can be easily blocked using apoB-containing lipoproteins (such as LDL) or with an LDL-receptor-specific antagonist monoclonal antibody [28,31]. Using the APP^{717}-transgenic mouse model to induce amyloid deposition with age, Holtzman *et al.* demonstrated that there was no amyloid deposition in the brains of knock-in mice in which the *apoE* gene had been replaced with human *apoE3* or *apoE4*, but that steady-state production of CNS β-amyloid was unaffected [33]. These results suggest that human apoEs (E3 or E4) exhibit physiological properties that are different from mouse apoE with regard to amyloid metabolism and to amyloid removal and/or deposition in the mature brain.

Although the role of apoE in the scavenging of β-amyloid in the brain is well documented, the exact mechanism by which cholesterol directly alters β-amyloid production is not entirely clear. Cholesterol exerts numerous effects on the APP secretase function (in addition to those reported on the apoE–LDL-receptor pathway). Increases in intracellular cholesterol concentrations lead to inhibition of α-secretase activity but stimulate β- and γ-secretase activities.

Recent evidence suggests that it might not be total cholesterol that regulates these secretase activities, but rather the ratio of free cholesterol (FC) to cholesterol esters (CE) [34]. In cells with increased amounts of CE, β-amyloid production is significantly increased, whereas cells that lack ACAT (the enzyme that converts FC to CE for storage purpose), and therefore have decreased levels of CE and increased intracellular FC, show decreased β-amyloid production. Furthermore, ACAT inhibitors decrease β-amyloid production in a variety of cells [34]. However, these effects are only observed when cells contain physiological or supra-physiological levels of FC, and not when cells exhibit low FC levels. These results are consistent with previous reports showing that extracellular addition of β-amyloid 1–40 or 1–42 to neuronal cell culture stimulates apoE-mediated cholesterol internalization [24], thus promoting a rise in intracellular FC. This, in turn, leads to inhibition of local β-amyloid production.

By contrast with treatments such as methyl-β-cyclodextrin, which alter total cholesterol, and reduce β- and γ-secretase activity and increase α-secretase activity, decreases in CE levels are normally accompanied by an apparent decrease in all three secretase activities [35].

Furthermore, cholesterol also appears to play a role in β aggregation, *in vitro* at least; *in vitro* assays have demonstrated that apoE, apoJ and/or cholesterol accelerate the formation of β aggregates whereas, *in vivo*, cholesterol is concentrated in the core of dendritic amyloid plaques in AD brains.

Several independent studies have shown that cholesterol-lowering agents, such as Probucol (which was used in the mid-1980s to reverse skin cholesterol deposition in familial hypercholesterolemia) and the statins, can alter β-amyloid immunoreactivity in animal models of amyloid deposition. In one study, guinea pigs receiving high doses (200 times that used in humans) of the HMGR inhibitor, simvastatin, for several weeks showed reduced levels of β-amyloid in the brain and cerebrospinal fluid (CSF) [36]. This compound, and other HMGR inhibitors, block the conversion of 3-hydroxy-3-methylglutaryl-coenzyme-A (HMG-CoA) to mevalonate, thereby decreasing cholesterol levels through inhibition of *de novo* synthesis. In the simvastatin-treated animals, serum cholesterol levels were reduced by 80% and, although brain cholesterol was not decreased, there was a twofold decrease in levels of the cholesterol precursor lanosterol. In another study, APP–PS-1 co-transgenic mice were chronically treated with the cholesterol synthesis inhibitor, BM15.766, a 7-dehydrocholesterol-reductase inhibitor that lowers cholesterol levels by inhibiting the conversion of lanosterol to cholesterol [37]. In this study, animals receiving the drug showed a significant reduction in β-amyloid levels and in plaque density.

Cholesterol metabolism: a potential therapeutic target for the treatment of sporadic AD

Cholesterol homeostasis in brain cells is maintained by the balance between cholesterol influx through the apoE–apoE-receptor pathway, cholesterol synthesis via the HMGR pathway, and cholesterol storage through esterification.

Cholesterol synthesis via the HMGR pathway (Figs 1 and 2) is normally required only when lipoprotein internalization via the apoE–apoE-receptor pathway is insufficient to meet the cholesterol requirement of the cell. The endoplasmic-reticulum-bound HMGR is regarded as the rate-limiting enzyme in the synthesis of cholesterol. The other (shorter) form of HMGR, localized in the peroxisomal compartment of embryonic cells, appears not to be important in cholesterol homeostasis, and is more resistant to HMGR inhibitors than its endoplasmic-reticulum counterpart [38]. The distribution of the peroxisomal form within the body has not yet been examined. In cells grown in an excess of cholesterol-rich lipoproteins, HMGR activity is downregulated in favor of uptake via apoE receptors. To maintain cellular cholesterol homeostasis, there is a potent negative feedback system that operates on the activity (Fig. 1) and gene expression of *HMGR*, resulting in decreased synthesis of cholesterol in response to sterol internalization via the apoE-receptor family. The most important feedback regulation of HMGR activity is through decreased gene transcription [39], although controlled degradation of the HMGR protein also occurs [40]. There is also evidence from hamster of modulation of HMGR mRNA translation efficiency, resulting in decreased or increased reductase protein and activity.

Evidence obtained from epidemiological studies indicates that the treatment of middle-aged subjects with statins confers protection against sporadic AD later in life [41–43], thus providing an interesting biochemical target. Treatment with these compounds leads to a reduction in the formation of LDL cholesterol and in its entry into the circulation, and an upregulation of LDL-receptor activity at the cell surface. Furthermore, serum LDL cholesterol and triglycerides are reduced, and HDL cholesterol is increased in the periphery. It is unclear whether the beneficial effects of statins require blood–brain penetration or are mediated through plasma cholesterol alterations. Inhibition of HMGR by statins is known to cause concomitant activation of the isoprenoid pathway, which is involved in coordination of the cell-cycle and in apoptosis. However, activation of this pathway by statins in the brain could irremediably activate an apoptotic cascade, which would damage terminally differentiated neurons rather them save them from AD.

The recent findings that treatment with statins helps to reduce the risk of developing AD [41–43] are similar to those reported for these drugs in other conditions, such as myocardial infarction and stroke, for which statins improve prognosis and survival beyond a measured effect on cholesterol concentrations. The effect of statins on the onset of AD is also consistent with a recent report on Probucol, a cholesterol-lowering agent that does not interfere with HMGR activity. This 'proof-of-principle' clinical study, which examined the effect of a standard dose of Probucol on mild–moderate AD, revealed a concomitant stabilization of symptoms, according to the Alzheimer's Disease Assessment Scale – Cognition (ADAS-Cog) and on the Disability Assessment of Dementia scale [44]. The clinical benefits on the ADAS-Cog correlated well with the increase in apoE levels in the CSF of these patients. Moreover, there was an inverse relationship between the apoE levels and the total β-amyloid levels in the CSF of

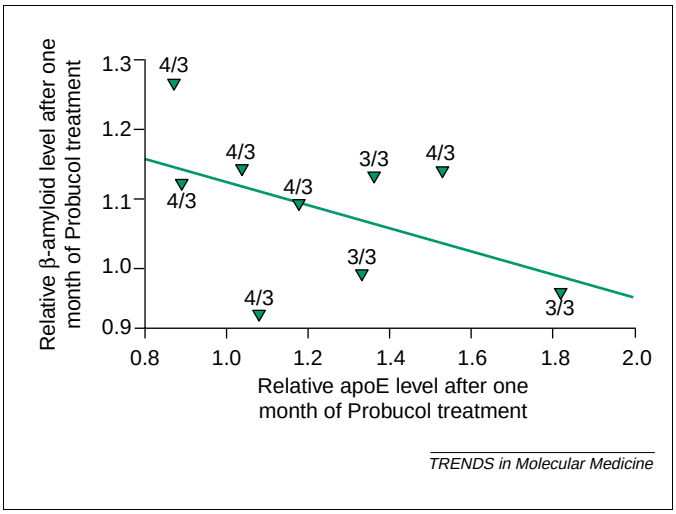

Fig. 3. Total β-amyloid levels in the cerebrospinal fluid of Alzheimer's disease (AD) patients treated with the cholesterol-lowering agent, Probucol. Correlational analysis of alterations in total β-amyloid levels as a function of apolipoprotein E (apoE) levels, measured in the cerebrospinal fluid of AD subjects after one month of Probucol treatment, relative to levels before the treatment began ($p < 0.05$) [13]. *ApoE* genotypes are presented for each subject (*apoE3/3* is abbreviated to 3/3 and *apoE4/3* to 4/3).

the Probucol-treated AD subjects (1 g/day for four weeks) (Fig. 3) [13].

These results are consistent with the findings from a recent study by Friedhoff and colleagues, who have used a generic version of lovastatin, a potent HMGR inhibitor that exhibits marked cholesterol-lowering effects, to reduce circulating levels of β-amyloid significantly in AD subjects enrolled in a six-month clinical drug trial [45]. The impact of lovastatin on clinical parameters has not yet been made public but if the effect is similar to Probucol a relative stabilization of the symptoms over the course of 6–12 months might be expected.

These independent and complementary results indicate that it might be possible to interfere with disease onset and/or progression in subjects exposed to cholesterol-lowering agents (statins or Probucol) before or following a diagnosis of Alzheimer's disease. It is still unclear whether the effect is specific to dementia of the Alzheimer type or is more general to other types of dementia, including vascular forms.

It is possible that the beneficial effect of cholesterol-lowering agents stems from a completely distinct pathway, involving an independent cardiovascular risk factor that normally modulates disease onset in 'at risk' subjects. In this scenario, cholesterol-lowering agents would act indirectly to prevent the effect of vascular risk factors, such as circulating levels of cholesterol or atherosclerotic plaque deposition, from modulating the age of onset in AD. Although it remains difficult to determine how cholesterol-lowering agents affect the pathophysiology of AD, recent findings showing the presence of polymorphic genetic variants in the *HMGR* gene [13] provide us with a possible

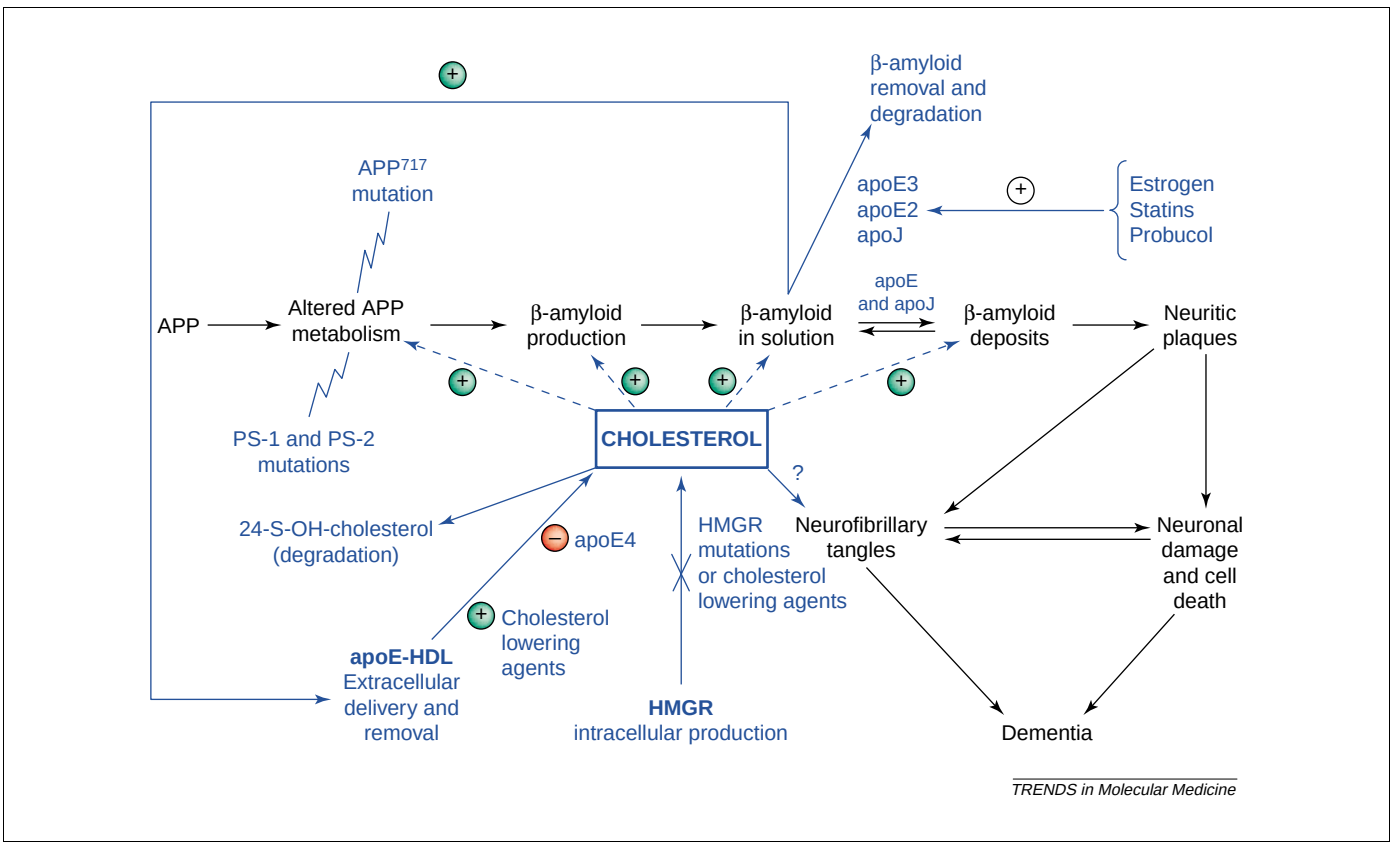

Fig. 4. A cholesterol–amyloid hypothesis of Alzheimer's disease (AD) pathophysiology. The sequence of pathogenic events leading to neuronal cell loss and synaptic damage is based on the well-established amyloid-cascade hypothesis, which proposes that accumulation of β-amyloid in the brain is the primary influence that drives AD pathology. The different modulators of β-amyloid metabolism that have been shown to affect lipid homeostasis, such as apolipoproteins apoE and apoJ, have been added to the cascade. The emerging contributions of cholesterol and 3-hydroxy-3-methylglutaryl-coenzyme-A reductase (HMGR) to the pathophysiology of AD have also been included. Abbreviations: APP, amyloid precursor protein; PS, presenilin.

explanation of the molecular basis and a target for the beneficial effect against AD.

Is there a unifying hypothesis of Alzheimer's disease pathophysiology?

Although age is key determinant of the expression of AD pathology, genetic risk factors also appear to play a central role in this process. Genes such as those encoding apoE4, LRP, the very-low-density-lipoprotein receptor, lipoprotein lipase and paraoxonase are all known modulators of cholesterol homeostasis that are also genetic risk factors for AD. The contribution of each key biochemical pathway involved in the pathophysiology of AD can be linked under one unifying hypothesis (Fig. 4), based on the original amyloid hypothesis of Hardy and Higgins [46]. This model also incorporates the physiologically relevant contribution of apoE [47] and HMGR [13], and highlighting the possible link between cholesterol metabolism and APP metabolism in AD [26].

Conclusions

Understanding the etiopathology of AD requires recognition of the interplay between the cardiovascular system, amyloid metabolism and the compensatory response of the brain to neuronal cell loss and synaptic damage. Pharmacological manipulations designed to increase apoE synthesis and secretion in the brain (irrespective of the *apoE* isotype) might prove useful in restoring lipid homeostasis in AD subjects, in promoting synaptic plasticity and regeneration, and in scavenging extracellular β-amyloid in areas of ongoing deafferentation [44]. Experimental or pharmacological manipulations of cholesterol synthesis *in situ* might offer similar benefits by indirectly affecting the apoE cascade or by directly affecting abnormal forms of the HMGR enzyme produced in the brain of AD subjects [13].

References

1 Tanzi, R.E. and Bertram, L. (2001) New frontiers in Alzheimer's disease genetics. *Neuron* 32, 181–184
2 Poirier, J. *et al.* (1993) Apolipoprotein E polymorphism and Alzheimer's disease. *Lancet* 342, 697–699
3 Strittmatter, W.J. *et al.* (1993) Apolipoprotein E: high-avidity binding to β-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc. Natl. Acad. Sci. U. S. A.* 90, 1977–1981
4 Kalaria, R.N. and Ballard, C. (1999) Overlap between pathology of Alzheimer disease and vascular dementia. *Alzheimer Dis. Assoc. Disord.* 13 (Suppl. 3), S115–S123
5 de Leon, M.J. *et al.* (2001) Prediction of cognitive decline in normal elderly subjects with 2-[^{18}F]fluoro-2-deoxy-D-glucose/positron-emission tomography (FDG/PET). *Proc. Natl. Acad. Sci. U. S. A.* 98, 10966–10971
6 Reiman, E.M. *et al.* (2001) Declining brain activity in cognitively normal apolipoprotein E ϵ4 heterozygotes: a foundation for using positron emission tomography to efficiently test treatments to prevent Alzheimer's disease. *Proc. Natl. Acad. Sci. U. S. A.* 98, 3334–3339
7 Skoog, I. (2000) Vascular aspects in Alzheimer's disease. *J. Neural Transm. Suppl.* 59, 37–43
8 Luchsinger, J.A. *et al.* (2002) Caloric intake and the risk of Alzheimer disease. *Arch. Neurol.* 59, 1258–1263
9 Peila, R. *et al.* (2001) Joint effect of the *APOE* gene and midlife systolic blood pressure on late-life cognitive impairment: the Honolulu–Asia aging study. *Stroke* 32, 2882–2889
10 Mulnard, R.A. *et al.* (2000) Estrogen replacement therapy for treatment of mild to moderate Alzheimer disease: a randomized controlled trial. Alzheimer's disease cooperative study. *JAMA* 283, 1007–1015
11 Distl, R. *et al.* (2001) Tangle-bearing neurons contain more free cholesterol than adjacent tangle-free neurons. *Acta Neuropathol. (Berl.)* 101, 547–554
12 Mulder, M. *et al.* (1998) Reduced levels of cholesterol, phospholipids, and fatty acids in cerebrospinal fluid of Alzheimer disease patients are not related to apolipoprotein E4. *Alzheimer Dis. Assoc. Disord.* 12, 198–203
13 Poirier, J. (2002) Cholesterol transport and synthesis are compromised in the brain in sporadic Alzheimer's disease: From risk factors to therapeutic targets. In *Alzheimer's Disease and Related Disorders Annual* (Gauthier, S. and Cummings, J.L., eds) pp. 1–23, Dunitz
14 Svennerholm, L. and Gottfries, C.G. (1994) Membrane lipids, selectively diminished in Alzheimer brains, suggest synapse loss as a primary event in early-onset form (type I) and demyelination in late-onset form (type II). *J. Neurochem.* 62, 1039–1047
15 Mason, R.P. *et al.* (1992) Evidence for changes in the Alzheimer's disease brain cortical membrane structure mediated by cholesterol. *Neurobiol. Aging* 13, 413–419
16 Giubilei, F. *et al.* (1990) Serum lipoprotein pattern variations in dementia and ischemic stroke. *Acta Neurol. Scand.* 81, 84–86
17 Jarvik, G.P. *et al.* (1995) Interactions of apolipoprotein E genotype, total cholesterol level, age, and sex in prediction of Alzheimer's disease: a case-control study. *Neurology* 45, 1092–1096
18 Roher, A.E. *et al.* (1999) Amyloid and lipids in the pathology of Alzheimer disease. *Amyloid* 6, 136–145
19 Kehoe, P. *et al.* (1999) A full genome scan for late onset Alzheimer's disease. *Hum. Mol. Genet.* 8, 237–245
20 Yasojima, K. *et al.* (2001) 3-hydroxy-3-methylglutaryl-coenzyme A reductase mRNA in Alzheimer and control brain. *Neuroreport* 12, 2935–2938
21 Poirier, J. *et al.* (1993) Cholesterol synthesis and lipoprotein reuptake during synaptic remodelling in hippocampus in adult rats. *Neuroscience* 55, 81–90
22 Nathan, B.P. *et al.* (1994) Differential effects of apolipoproteins E3 and E4 on neuronal growth *in vitro*. *Science* 264, 850–852
23 Poirier, J. *et al.* (1995) Apolipoprotein E, synaptic plasticity and Alzheimer's disease. *Ann. Med.* 27, 663–670
24 Beffert, U. *et al.* (1996) Apolipoprotein E uptake is increased by β-amyloid peptides and reduced by blockade of the LDL receptor. *Mol. Brain Res.* 68, 181–185
25 Sliskovic, D.R. and White, A.D. (1991) Therapeutic potential of ACAT inhibitors as lipid lowering and anti-atherosclerotic agents. *Trends Pharmacol. Sci.* 12, 194–199
26 Sparks, D.L. *et al.* (1994) Induction of Alzheimer-like β-amyloid immunoreactivity in the brains of rabbits with dietary cholesterol. *Exp. Neurol.* 126, 88–94
27 Schmechel, D. and Sullivan, P. (2002) High saturated fat diets are associated with a β deposition in primates. *Neurobiol. Aging* 23, 323
28 Refolo, L.M. *et al.* (2000) Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model. *Neurobiol. Dis.* 7, 321–331
29 Shie, F.S. *et al.* (2002) Diet-induced hypercholesterolemia enhances brain Aβ accumulation in transgenic mice. *Neuroreport* 13, 455–459
30 Howland, D.S. *et al.* (1998) Modulation of secreted β-amyloid precursor protein and amyloid β-peptide in brain by cholesterol. *J. Biol. Chem.* 273, 16576–16582
31 Beffert, U. and Poirier, J. (1996) Apolipoprotein E, plaques, tangles and cholinergic dysfunction in Alzheimer's disease. *Ann. New York Acad. Sci.* 777, 166–174
32 Beffert, U. *et al.* (1999) Apolipoprotein E isoform-specific reduction of extracellular amyloid in neuronal cultures. *Mol. Brain Res.* 68, 181–185
33 Holtzman, D.M. *et al.* (1999) Expression of human apolipoprotein E reduces amyloid-β deposition in a mouse model of Alzheimer's disease. *J. Clin. Invest.* 103, R15–R21
34 Puglielli, L. *et al.* (2001) Acyl-coenzyme A: cholesterol acyltransferase modulates the generation of the amyloid β-peptide. *Nat. Cell Biol.* 3, 905–912
35 Bodovitz, S. and Klein, W.L. (1996) Cholesterol modulates α-secretase cleavage of amyloid precursor protein. *J. Biol. Chem.* 271, 4436–4440

36 Fassbender, K. *et al.* (2001) Simvastatin strongly reduces levels of Alzheimer's disease β-amyloid peptides Aβ42 and Aβ40 *in vitro* and *in vivo*. *Proc. Natl. Acad. Sci. U. S. A.* 98, 5856–5861
37 Refolo, L.M. *et al.* (2001) A cholesterol-lowering drug reduces β-amyloid pathology in a transgenic mouse model of Alzheimer's disease. *Neurobiol. Dis.* 8, 890–899
38 Aboushadi, N. *et al.* (2000) Characterization of peroxisomal 3-hydroxy-3-methylglutaryl coenzyme A reductase in UT2 cells: sterol biosynthesis, phosphorylation, degradation, and statin inhibition. *Biochemistry* 39, 237–247
39 Reynolds, G.A. *et al.* (1984) HMG CoA reductase: a negatively regulated gene with unusual promoter and 5′ untranslated regions. *Cell* 38, 275–285
40 Gardner, R.G. and Hampton, R.Y. (1999) A highly conserved signal controls degradation of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase in eukaryotes. *J. Biol. Chem.* 274, 31671–31678
41 Jick, H. *et al.* (2000) Statins and the risk of dementia. *Lancet* 356, 1627–1631
42 Rockwood, K. *et al.* (2002) Use of lipid-lowering agents, indication bias, and the risk of dementia in community-dwelling elderly people. *Arch. Neurol.* 59, 223–227
43 Wolozin, B. *et al.* (2000) Decreased prevalence of Alzheimer disease associated with 3-hydroxy-3-methyglutaryl coenzyme A reductase inhibitors. *Arch. Neurol.* 57, 1439–1443
44 Poirier, J. and Panisset, M. (2002) Apolipoprotein E: A novel therapeutic target for the treatment of Alzheimer's Disease. *Adv. Behav. Biol.* 1, 36–42
45 Friedhoff, L.T. *et al.* (2001) Treatment with controlled-release lovastatin decreases serum concentrations of human β-amyloid (Aβ) peptide. *Int. J. Neuropsychopharmacol.* 4, 127–130
46 Hardy, J.A. and Higgins, G.A. (1992) Alzheimer's disease: the amyloid cascade hypothesis. *Science* 256, 184–185
47 Poirier, J. (1994) Apolipoprotein E in animal models of CNS injury and in Alzheimer's disease. *Trends Neurosci.* 17, 525–530