

Alzheimer's disease: the cholesterol connection

Luigi Puglielli^{1,2}, Rudolph E. Tanzi² and Dora M. Kovacs^{1,2}

¹Neurobiology of Disease Laboratory and ²Genetics and Aging Research Unit, CAGN, Massachusetts General Hospital, Harvard Medical School, Charlestown, Massachusetts 02129, USA

Correspondence should be addressed to D.M.K. (kovacs@helix.mgh.harvard.edu)

A hallmark of all forms of Alzheimer's disease (AD) is an abnormal accumulation of the β -amyloid protein (A β) in specific brain regions. Both the generation and clearance of A β are regulated by cholesterol. Elevated cholesterol levels increase A β in cellular and most animals models of AD, and drugs that inhibit cholesterol synthesis lower A β in these models. Recent studies show that not only the total amount, but also the distribution of cholesterol within neurons, impacts A β biogenesis. The identification of a variant of the apolipoprotein E (APOE) gene as a major genetic risk factor for AD is also consistent with a role for cholesterol in the pathogenesis of AD. Clinical trials have recently been initiated to test whether lowering plasma and/or neuronal cholesterol levels is a viable strategy for treating and preventing AD. In this review, we describe recent findings concerning the molecular mechanisms underlying the cholesterol-AD connection.

Alzheimer's disease (AD) is the most common form of dementia, affecting up to 15 million individuals worldwide. Because of the ongoing increase in life expectancy, by 2050 we can expect approximately 25% of people living in the Western hemisphere to be over 65 years of age, one third of whom are likely to develop AD. AD is a complex and genetically heterogeneous disease, characterized by progressive memory deficit, cognitive impairment and personality changes accompanied by specific structural abnormalities in the brain.

The main histological features of AD are extracellular protein deposits termed β -amyloid (or senile) plaques, β -amyloid deposits in blood vessels and intraneuronal neurofibrillary tangles. Loss of neurons and synapses in the neocortex, hippocampus and other subcortical regions of the brain are also common features. High numbers of amyloid plaques in limbic and association cortices serve as the basis for a definitive post-mortem diagnosis of AD. The chief component of the plaque core is the ~4 kDa amyloid β -peptide (A β) organized in 7–10 nm fibrils intermixed with non-fibrillar forms of the peptide.

ApoE is a 299 amino acid glycoprotein and the major protein component of very low-density lipoproteins (VLDL). ApoE is also the major apolipoprotein in the brain. The identification of the ϵ 4 variant of APOE as the most common genetic risk factor for late-onset AD suggests that cholesterol may play a direct role in the pathogenesis of the disease. Epidemiological, molecular and biochemical evidence have further strengthened the hypothesis that cholesterol is a risk factor for AD. Although cholesterol homeostasis in the brain is largely unexplored, new findings strongly support the involvement of cholesterol in both the generation and deposition of A β .

Genetics and biology of AD

The genetics of AD is best explained by an age-dependent model involving either rare, early-onset (<60 years old) causative mutations or late-onset (>60 years old) genetic risk factors that increase the risk of developing the disease (for review, see ref. 1).

Early-onset familial AD (FAD) has so far been linked to mutations in the genes for the β -amyloid precursor protein (APP) on chromosome 21, presenilin 1 (PSEN1) on chromosome 14, and presenilin 2 (PSEN2) on chromosome 1. Together, they are thought to account for ~40% of early-onset AD (for review, see refs. 1,2). Late-onset AD accounts for ~95% of AD cases. Late-onset AD genes implicated thus far harbor genetic polymorphisms that act as either risk factors and/or genetic modifiers. Although over three dozen putative genetic risk factors for AD have been reported in the literature, only the ϵ 4 allele of the APOE gene on chromosome 19 has been consistently found associated with AD in several independent studies (for review, see ref. 1). Additional genetic loci for late-onset AD have been localized on chromosomes 9 and 10 (for review, see ref. 1), as well as other chromosomes³.

The common pathogenic event that occurs in all forms of AD is the abnormal accumulation of A β in amyloid deposits and cerebral blood vessels. In most forms of early-onset FAD, this correlates with increased production of the 42-amino acid isoform of A β (A β ₄₂), which promotes the aggregation of total A β into amyloid fibrils. These patients develop AD pathology by middle age. In contrast to early-onset FAD, the accumulation of A β in late-onset AD is the result of a complex interplay between genetic and environmental factors, affecting generation, clearance and aggregation of the peptide (for review, see ref. 4).

Several isoforms of the APP protein are expressed, APP₆₉₅ being the most common form expressed in the brain, and originate from alternative splicing of a single gene, APP. A β is generated by an initial cleavage of APP at the N-terminus by a beta secretase (BACE, which stands for β -site APP cleaving enzyme), and then in the transmembrane domain by a γ -secretase, a multimeric protein complex that includes presenilin (Fig. 1). The two major sites of γ -cleavage are located at positions 40 and 42 of A β , generating A β ₄₀ and A β ₄₂, respectively.

The physiologic role of APP still remains elusive (for review, see ref. 5). Secreted APP isoforms containing the KPI domain

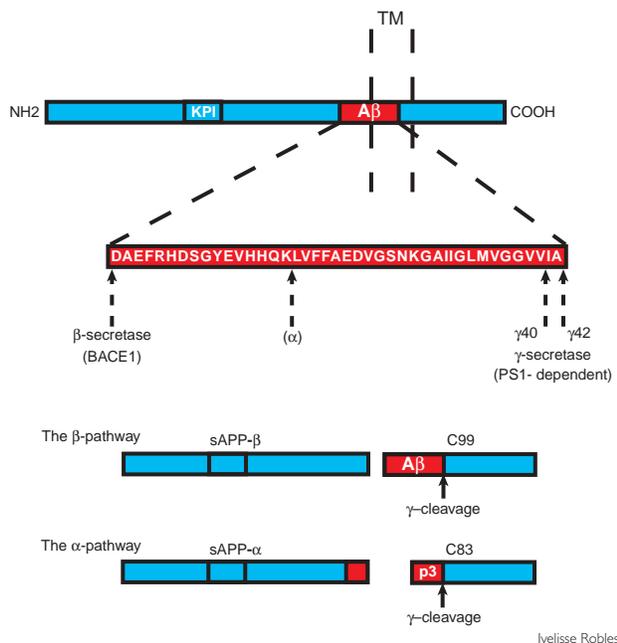


Fig. 1. Proteolytic processing of APP. APP is a type-I glycoprotein with its amino terminus on the luminal/extracellular surface and a short C-terminal cytoplasmic tail. The major component of amyloid plaques, the Aβ peptide (shown in red), is produced by the β pathway, where APP is first cleaved at the N-terminus of Aβ (β-cleavage) and then in the transmembrane domain (γ-cleavage), either at position 40 or 42. In contrast, APP is more frequently cleaved at the α position, between amino acids 16 and 17 of the Aβ region, precluding the generation of Aβ. The APP C-terminal fragments (APP-CTFs) produced after α (α-APP-CTF) and β cleavage (β-APP-CTF) of APP are also respectively called C-83 and C-99, based on the number of amino acids.

Although controversial, several *in vitro* and *in vivo* studies have shown that apoE can bind Aβ^{11,12}. Methodological problems have made it difficult to draw firm conclusions on the role of lipid-free or lipid-associated apoE in Aβ aggregation, and the pathophysiological importance of this phenomenon is not clear. Nevertheless, some reports indicate that apoE-mediated binding of Aβ to the cell surface could be isoform-specific: E2>E3>E4 (refs. 12,13). ApoE may also mediate internalization of Aβ through its binding to the LDL receptor-related protein^{11,14–17} (LRP; see Box 1 and Fig. 2).

Recent studies also show that Aβ internalization is not necessarily followed by its degradation. Instead, Aβ aggregates in the endocytic compartment and can be secreted again in the fibrillar and more toxic form^{14,15}. Thus, apoE may ultimately contribute to Aβ aggregation by mediating its internalization (Fig. 2, Model I). In addition, lipid-free apoE4 has been shown to more effectively bind Aβ¹⁸ and promote Aβ fibril formation than the apoE3 isoform^{19,20}. One possible scenario is that apoE could promote the aggregation of Aβ in the endosomal compartment subsequent to receptor-mediated internalization and enzymatic digestion of the lipid particle, which renders apoE lipid-free and able to interact with Aβ. This conclusion would be supported by the facilitation of Aβ fibril formation observed in AD transgenic mice expressing the apoE4 isoform^{10,21,22}.

In addition to directly facilitating Aβ internalization and aggregation, apoE4 may also alter brain cholesterol homeostasis by modifying lipoprotein-particle formation. In the plasma, apoE4 tends to associate with VLDL particles, which contain more cholesterol, whereas apoE3 prefers to associate with HDL. Subjects homozygous for the APOE ε4 allele have higher levels of cholesterol in the plasma^{23,24} and 24S-hydroxycholesterol in the cerebrospinal fluid (CSF)²⁵. 24S-hydroxycholesterol is a catabolic derivative of cholesterol and represents the major metabolic route for cholesterol clearance from the brain. The lipoproteins produced in the brain are very different in virtually all respects (composition, density and other properties) from the particles found in the plasma (for review, see ref. 21). As apoE4 shows differential preference for VLDL particles in the plasma, it is also conceivable that different apoE isoforms modify brain cholesterol homeostasis by preferentially associating with specific lipoprotein particles. The predominant nature of apoE in brain lipoproteins would accentuate small differences in lipoprotein affinity for apoE isoforms. Therefore, the role of apoE in maintaining cholesterol homeostasis in the brain may contribute to the increased risk for AD associated with APOE ε4 (Model II in Fig. 2).

A number of epidemiological studies suggest that high levels of cholesterol may contribute to the pathogenesis of AD. Individuals with elevated levels of plasma cholesterol have an increased susceptibility to AD, apparently influenced by the APOE ε4 genotype^{26,27}. Moreover, AD patients have increased levels of total serum and low-density lipoprotein (LDL) cholesterol^{26,27} along

(Kunitz-type serine protease inhibitors) may function in the coagulation pathway⁵. APP's cytoplasmic domain has been shown to interact with several cytosolic adaptor proteins and can function as a signaling molecule^{6,7}, but a putative extracellular ligand has not yet been identified.

Cholesterol and AD: apoE and epidemiology

ApoE is one of the major apolipoproteins in the plasma and the principal cholesterol carrier protein in the brain. In humans, there are three common alleles of the APOE gene: ε2, ε3 and ε4. The protein isoforms produced by these alleles differ in the amino acids at positions 112 and/or 158: E2 (Cys112, Cys158), E3 (Cys112, Arg158), which is the most common, and E4 (Arg112, Arg158), which is present in at least one copy in ~25% of the population. Numerous independent studies have consistently confirmed that the APOE ε4 allele is the most prevalent risk factor for AD (for review, see ref. 1). The risk for AD conferred by APOE ε4 increases in a dose-dependent manner; individuals that are homozygous for APOE ε4 alleles (~2% of the population) are eight times more likely to develop AD than are homozygotes for APOE ε3. However, APOE ε4 is neither necessary nor sufficient to cause AD; it only increases risk for the disease¹.

The association between AD and APOE ε4 remains largely undefined at the mechanistic level. Studies in a transgenic mouse model expressing human APP^{V717F+/-} (PDAPP mice) suggest that apoE contributes to the deposition of Aβ. Disruption of the APOE gene in PDAPP mice inhibited the accumulation of Aβ in immunoreactive deposits⁸. Moreover, amyloid deposition was found to be strictly dependent on apoE expression levels in a dose-dependent manner: PDAPP mice hemizygous for apoE were found to have a ~60% reduction in amyloid deposition, whereas no nonfibrillar or fibrillar Aβ was detected in any brain region of homozygous knockout mice. The absence of apoE was shown to affect the extracellular accumulation, but not the synthesis, of Aβ⁹. Re-introduction of human APOE genes in the APP^{V717F+/-}, APOE^{-/-} mice restored deposition of fibrillar Aβ¹⁰. Deposition of Aβ associated with severe neuritic dystrophy was more evident with the APOE ε4 isoform.

Box 1. LRP

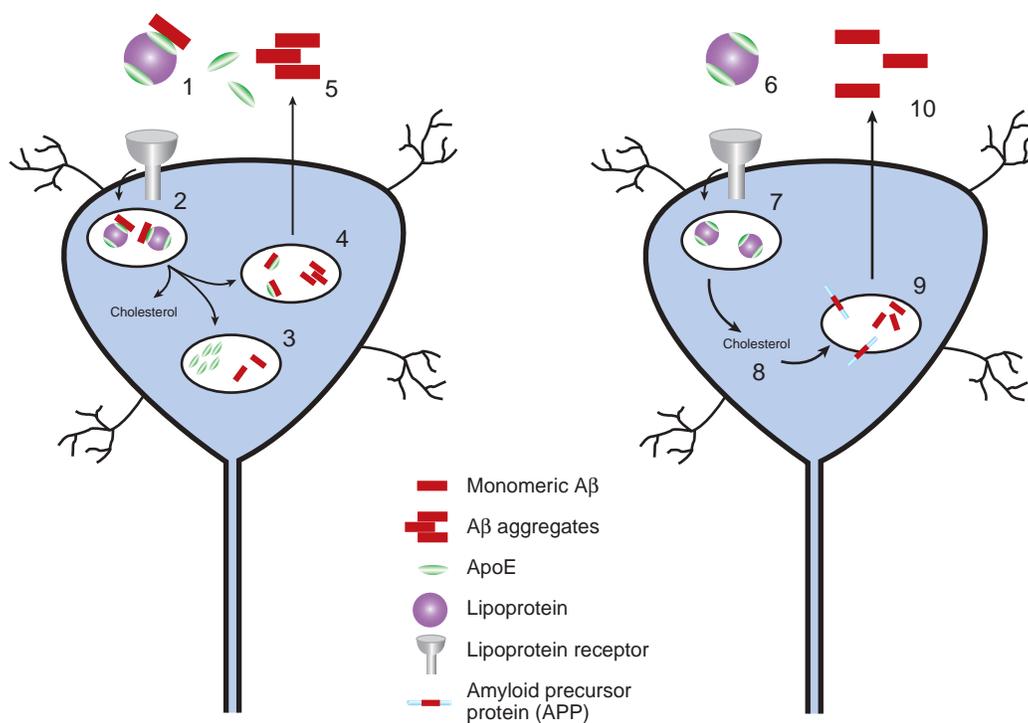
LRP is a multi-ligand receptor and a member of the LDL receptor (LDLr) family. With the LDLr it shares common structural motifs and high-affinity binding activity for LDL particles. LRP's main protein ligand is apoE, but it also binds other molecules such as α 2-macroglobulin and secreted APP_{751/770} containing the KPI domain (for review, see ref. 17). LRP may be linked to the degradation of secreted A β by facilitating the internalization of A β bound to apoE¹⁴. Recent studies, however, have shown that LRP-mediated internalization of A β is followed by its intracellular aggregation, rather than degradation, suggesting that LRP might be involved in the process of A β deposition¹⁵ (Fig. 2). LRP not only mediates the internalization of A β , but also its production. The interaction of LRP with the KPI domain of APP_{751/770} increases the generation of A β ¹⁶.

with reduced levels of apoA/high-density lipoprotein (HDL) in their plasma^{27,28}, as compared to age-matched controls. This is further strengthened by the fact that lecithin cholesterol acyltransferase (LCAT) activity is significantly decreased in AD patients²⁹. LCAT is an enzyme found in plasma that catalyzes an acyltransferase reaction on lipoprotein-associated cholesterol and is a key step in reverse cholesterol transport in humans (the process that eliminates cholesterol from peripheral cells). This metabolic profile (high plasma cholesterol with high LDL-cholesterol and low HDL-cholesterol) is commonly found in patients with atherosclerosis. Also, atherosclerosis, intimately related to high blood cholesterol, has been shown to correlate with an increased risk of AD, with higher levels of risk being associated with advanced atherosclerosis³⁰. As mentioned above, AD patients in early stages of dementia show increased levels of 24S-

hydroxycholesterol in the CSF²⁵. Finally, cholesterol abnormally accumulates in the dense cores of amyloid plaques in the brain of AD patients³¹. Similar accumulation of cholesterol has also been found in amyloid plaques of transgenic mice expressing a mutant form of APP₆₉₅ (Swedish mutation) associated with FAD³¹.

Cholesterol in A β generation and deposition

Two initial reports indicated that statins, which reduce serum levels of cholesterol, also protect against AD^{32,33}. Although both studies were retrospective (data obtained from past hospital records) in nature, the results already prompted the initiation of clinical trials for the use of statins in AD. In one of these initial studies³², the protective effect of statins was independent of the presence or absence of untreated high lipid levels, suggesting that statins may protect against AD through an unknown mechanism



Ivelisse Robles

Fig. 2. Two possible models for apoE's role in A β accumulation. **Model I:** Soluble A β interacts with apoE associated with a lipoparticle (1) and undergoes receptor-mediated endocytosis. Lipoproteins are then enzymatically digested in the lysosomal compartment (2), releasing cholesterol to the cell. In the lysosomes, a fraction of apoE and A β undergoes degradation (3), while the rest of apoE remains associated with A β and promotes its aggregation into amyloid fibrils (4), and is then secreted back into the extracellular milieu (5). Given that apoE4 has more affinity for A β than apoE2 and apoE3, it would be expected to accelerate this process. **Model II:** In addition to directly facilitating A β internalization and aggregation, apoE may also up-regulate the rate of A β generation by increasing cellular cholesterol. After receptor-mediated internalization (6) and enzymatic digestion of the lipoproteins (7), cholesterol is released to cellular membranes (8). ApoE4 lipoproteins tend to contain more cholesterol. The increased sterol content of intracellular membranes promotes the rate of A β generation (from its precursor APP) (9), resulting in increased secretion into the extracellular milieu (10).

not directly related to cholesterol. In the other³³, lovastatin and pravastatin reduced the risk of AD up to 73%. Despite the preliminary nature of these studies' conclusions, they readily corroborate pre-existing epidemiological data, and were subsequently confirmed by similar studies conducted in independent sets of patients^{33–35}. Interestingly, recent results from the first 26-week randomized study in 44 AD patients with normal cholesterol showed that simvastatin was able to reduce A β ₄₀ levels in the cerebrospinal fluid of patients with mild, but not severe, AD³⁶. A slight improvement in cognitive function reported in this study was not confirmed by a second randomized controlled trial on a much larger scale (called PROSPER for "pravastatin in elderly individuals at risk of vascular disease")³⁷. However, patients enrolled in the PROSPER trial were not screened for AD, and the trial included individuals with high cholesterol levels. Results from additional ongoing randomized controlled trials should soon be available and will allow for definitive conclusions on the possible use of statins against AD. Finally, it should be pointed out that the effects of statins on A β deposition in the brain may be independent of the changes in the CNS cholesterol metabolism³⁸, and may instead involve anti-inflammatory effects of statins³⁹ or alterations in the cycling of A β between the brain and plasma.

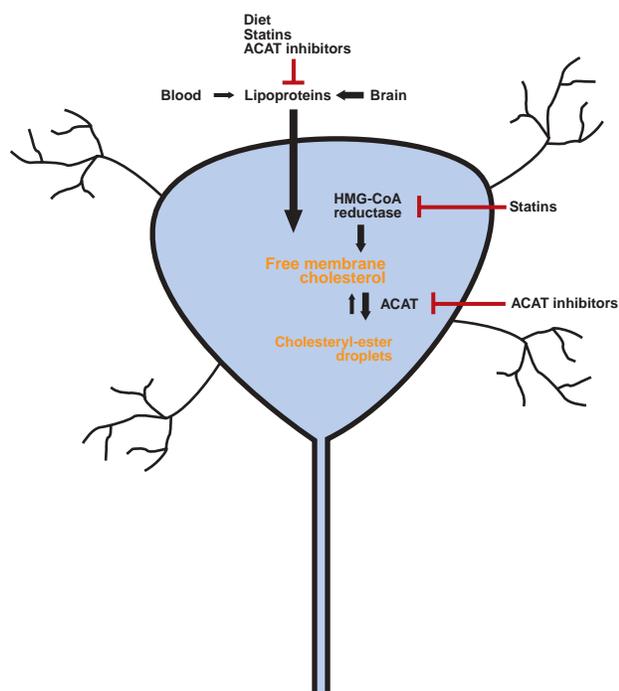
Studies using animal models of AD, including rabbits^{40,41}, transgenic mice^{42–44} and guinea pigs⁴⁵, show a strong connection between plasma cholesterol levels and A β generation. A recent report shows that a high fat/high cholesterol diet raises cholesterol levels in plasma and CNS of transgenic mice expressing the FAD mutants APP_{K670N,M671L} and PS1_{M146V}⁴². Because both β -APP C-terminal fragment (CTF) and A β levels were increased in the brain of these animals, the authors concluded that cholesterol levels can regulate APP processing and A β generation *in vivo*, possibly by a mechanism shown in Fig. 2 (Model II). Neuropathological analysis showed that a high cholesterol diet also increased the deposition of amyloid plaques. Additionally, cholesterol-lowering agents reversed the effect of high fat/high cholesterol diet on A β accumulation and cholesterol levels in the plasma and CNS⁴³. In a similar study on mice harboring a different APP FAD mutation (Swedish mutant), the high-cholesterol diet also elevated cholesterol levels in the plasma and CNS, but had an opposite effect on A β generation⁴⁴. In this model, the levels of both A β ₄₀ and A β ₄₂ were reduced in the brain. The reason for the apparent discrepancy may reside in either the genetic background of the mice or, more likely, in the different transgene introduced. Species differences in mice may affect cholesterol generation, and therefore the choice of APP transgenic mouse model used becomes extremely important for the outcome of studies in which cholesterol levels are manipulated. A recent study in guinea pigs has strengthened the role of lipid-lowering drugs in the regulation of A β generation, as simvastatin reduced cholesterol and both A β ₄₀ and A β ₄₂ levels in guinea pig plasma⁴⁵.

In addition to the *in vivo* results from animal studies, strong biochemical evidence also supports a direct role of intracellular cholesterol in A β generation and deposition. First, both APP and A β are associated, at least in part, with cholesterol-rich domains (CRDs)^{46,47}, specialized membrane micro-domains characterized by the tight association of cholesterol, sphingomyelin and highly-charged galactosphingolipids. CRDs serve as clustering domains for several membrane-bound proteins, and they have also been proposed to regulate APP processing by favoring the clustering of APP and BACE⁴⁸. Several studies have also reported a physical association of A β with GM1, the major galacto-sphingolipid in CRDs^{49,50}. This association facilitates

the aggregation of soluble A β in the form of amyloid fibrils⁵¹. CRDs may facilitate the aggregation of secreted A β , but their specific role in the processing of APP remains to be determined. Second, studies in primary neurons or peripheral cell lines in which total cholesterol content has been either reduced or increased indicate that cholesterol levels can actively regulate APP processing and A β generation. An increase in cholesterol up-regulates, whereas a decrease down-regulates, A β generation^{45,52,53}. Corresponding changes in the β -cleavage of APP suggest a direct effect of cholesterol on APP processing. In other studies, brief exposure to water-solubilized cholesterol reduced only the α -cleavage of APP⁵⁴. This was most likely due to transitory changes in cholesterol levels/distribution at the plasma membrane since the effect was independent of receptor-mediated internalization of cholesterol⁵⁵ and may involve regulated, but not necessarily constitutive, α -cleavage of APP⁵⁶. Finally, drugs that arrest cholesterol trafficking along the endocytic compartment affect the subcellular localization of PS1⁵⁷, whereas those that induce the expression of the cholesterol efflux receptor ABCA1 increase the secretion of A β ⁵⁸.

We have recently shown, through genetic, biochemical and metabolic approaches, that intracellular cholesterol distribution, rather than total cholesterol level, regulates APP processing and A β generation⁵⁹. Cellular cholesterol is stored either as free cholesterol in the membrane or as cholesteryl-esters in the form of cytoplasmic droplets (Fig. 3). An endoplasmic reticulum (ER) resident enzyme, acyl-coenzyme A cholesterol acyltransferase (ACAT), catalyzes the formation of cholesteryl-esters from cholesterol and long-chain fatty acids (Box 2; reviewed in refs. 60,61). ACAT controls the dynamic equilibrium between these two forms of cellular cholesterol, ultimately affecting cholesterol homeostasis (for review, see ref. 62). Our results indicate that this dynamic equilibrium ultimately regulates the generation of A β . A selective increase in cholesteryl-esters is sufficient to up-regulate the generation of A β and increase the steady-state levels of β -APP CTFs. We also showed that, in several cell lines and in primary neurons, ACAT competitive inhibitors reduce both cholesteryl-ester and A β biosynthesis in a dose-dependent manner, while increasing free cholesterol. Similar results were obtained with agents that block delivery of free cholesterol to ACAT, using water-solubilized cholesterol intermixed with LDL to radio-label intracellular cholesterol⁵⁷. Confirming evidence comes from *in vivo* studies, showing that a decrease in cholesteryl-ester levels in the brain of *Drosophila melanogaster* was associated with a vacuolar form of neurodegeneration and altered processing of fly APP⁶³. The fact that *Drosophila* brains harbor even fewer cholesteryl-esters than mammalian brains strongly indicates that very small amounts of this lipid are sufficient for its regulatory function in APP processing.

How cholesterol distribution affects the processing of APP is not yet clear. Some key questions remain unresolved. First, what is the specific contribution of cholesteryl-esters and free cholesterol? Whereas our previous findings implicate cholesteryl-esters, free cholesterol in cellular membranes also participates in the regulatory process because altered cholesteryl-ester levels modulate the free cholesterol pool. Even undetectable changes in free cholesterol levels may affect specific compartments involved in APP processing. Second, what are the molecular mechanisms involved in ACAT-mediated regulation of APP processing? Our results show that ACAT activity regulates all three major cleavages of APP. Thus, altered cholesterol distribution affects either the activity of all three secretases (α , β and γ), APP itself, or an as-yet-unidentified protein that controls APP processing. The



Melisse Robles

Fig. 3. Schematic view of cholesterol homeostasis in neurons and cholesterol-related targets for therapeutic treatment of AD. Neurons maintain cholesterol homeostasis mainly through *de novo* biosynthesis in the endoplasmic reticulum and receptor-mediated internalization of lipoproteins. The latter are either generated in the brain or, possibly, obtained from the plasma. This pool of free cholesterol constitutes the substrate of ACAT, which generates cholesteryl-esters and regulates A β generation. Free cholesterol available in the cell allosterically regulates ACAT activity. Statins, which inhibit hydroxy-methyl-glutaryl-CoA (HMG-CoA) reductase (the rate-limiting enzyme in cholesterol biosynthesis), act at two different levels. They reduce the neo-synthesis of cholesterol in the CNS (after crossing the blood–brain barrier), as well as levels of lipoprotein-associated cholesterol in the plasma. Cholesterol levels in the plasma can also be regulated by dietary restriction. Reduction of sterol content in neurons will affect ACAT activity and therefore the generation of cholesteryl-esters. Studies with animals indicate that such an approach is effective in regulating A β generation in the brain. ACAT activity can be directly regulated through specific competitive and non-competitive inhibitors, and may constitute a novel and more direct approach for the treatment and prevention of AD.

above findings, from our and other groups, clearly indicate that changes in cholesterol homeostasis, distribution and compartmentation impact both the processing of APP and the biogenesis of A β . The relevance of these data to brain A β generation remains to be confirmed. High levels of free cholesterol immobilized in the myelin membranes have, in the past, made quantitation of ‘mobile’ free cholesterol and cholesteryl-esters in the brain very difficult (discussed further below). Cell culture and transgenic animal studies on the effect of cholesterol on A β generation must be carried out along with more basic research to seek a better understanding of the mechanisms that govern cholesterol balance in the CNS and cholesterol metabolism in neurons.

Cholesterol metabolism in the brain

Overall, the brain is the organ with the highest content of cholesterol in the body, containing ~20% of total body cholesterol, but accounting for only ~2% of body mass (for review, see

Box 2. ACAT

ACAT is essential for the regulation of intracellular cholesterol homeostasis and distribution of cholesterol throughout the body. In the small intestine and liver it also regulates the secretion of chylomicrons and very large-density lipoproteins (VLDL), respectively. Mammals, including humans, express two different isoforms of ACAT, called ACAT-1 and ACAT-2. Whereas ACAT-1 is almost uniformly distributed among several tissues, including the brain, ACAT-2 is selectively expressed in the liver and intestine (for review, see refs. 60,61). Both ACAT-1 and ACAT-2 are ER resident enzymes, allosterically regulated by cholesterol available in the ER membrane. Elevated free cholesterol results in the activation of ACAT and production of cholesteryl-esters. A close relationship exists between ACAT, intracellular cholesterol trafficking, and the sterol regulatory element binding protein (SREBP) pathway, a complex group of membrane proteins that regulate cholesterol homeostasis⁶². ACAT keeps the levels of free cholesterol in the ER membrane under strict control. This pool of free cholesterol ultimately regulates the SREBP pathway, cholesterol trafficking and several molecular events in which cholesterol is directly involved, including gene regulation and expression, post-translational modifications and sorting of proteins, signal transduction and membrane trafficking.

ref. 64). Most brain cholesterol is unesterified (free) and is found within the specialized membranes of myelin⁶⁵. Since myelin has a very slow turnover rate, myelin-associated cholesterol is virtually immobilized. The remaining brain cholesterol is found in neurons, glial cells and extracellular lipoproteins, and these pools of cholesterol participate in cholesterol homeostasis of the CNS. However, the large mass of cholesterol sequestered into myelin membranes makes the analysis of cholesterol distribution in the brain technically challenging. Direct analysis of brain cholesterol metabolism is further complicated by the separation of brain cholesterol from plasma cholesterol owing to the blood–brain barrier.

Direct quantification of plasma cholesterol in the brain, which is very difficult, has thus far yielded negative results (for review, see refs. 64,65). Therefore, it is commonly assumed that all brain cholesterol originates from *in situ* neo-synthesis. This conclusion is based mainly on studies tracking the incorporation of tritiated water into the pool of sterols contained in the brain. Additionally, only small amounts of apoB, the main apolipoprotein associated with LDL particles, can be detected in the brain, arguing against significant amounts of full-size LDL particles passing the blood–brain barrier (for review, see refs. 64,65). A few studies suggesting that at least small amounts of cholesterol from the plasma may enter the CNS are also worthy of mention. First, brain cholesterol content is increased in hypercholesterolemic transgenic mice⁶⁶, and second, a high-lipid diet not only increases cholesterol levels in the plasma, but also the influx of sterols into the CNS^{42–44} (see also refs. 64 and 67 for review). The third line of evidence derives from observations obtained from patients affected by an inherited human disease, cerebrotendinous xanthomatosis. This disorder is caused by a metabolic defect in the biosynthesis of bile acids, which leads to an abnormal hepatic production of cholestanol (a 5 *c*-dihydro derivative of cholesterol)⁶⁸. Cholestanol abnormally accumulates in CNS of patients^{69,70}, indicating that LDL and/or VLDL can cross the adult blood–brain barrier into the CNS.

In summary, although conventional wisdom would predict that cholesterol homeostasis in the brain is not affected by plas-

ma cholesterol, there is growing evidence to the contrary. On the basis of these observations, cholesterol-lowering drugs as well as diet could become valid candidates for the therapeutic treatment and prevention of AD.

CONCLUSIONS

The past few years have witnessed the establishment of cholesterol as a *bona fide* risk factor in the pathogenesis of AD, supported by genetic, epidemiological and biochemical data. The application of this knowledge to the treatment and prevention of AD is already a major focus of current research. Drugs that lower cholesterol levels are currently being considered and tested as potential therapies for the treatment of AD (Fig. 3). Statins, which are relatively safe and have been used for a long time against hypercholesterolemia, are now being directly tested in clinical trials for efficacy against AD. Some of the potentially beneficial effects of statins might also represent improved cardiovascular health, resulting in a reduction in ischemic events that are also considered risk factors for AD. An effective therapy for patients whose cognitive function does not benefit from statin treatment may ultimately consist of a combination of lipid-regulating products, perhaps in combination with statins. Alternative products for cholesterol management so far include extended-release niacin, cholesterol absorption inhibitors, ACAT inhibitors and cholesteryl ester transfer protein (CETP) inhibitors. Results from *in vitro* studies suggest that ACAT inhibitors are good candidates for regulating A β biogenesis, but more research is needed to understand the exact molecular mechanisms underlying the AD–cholesterol connection.

Moreover, it is also necessary to gain an in-depth understanding of brain cholesterol metabolism. Although technical difficulties have been an obstacle for many studies, new technologies are rapidly emerging and should clarify the contribution of plasma cholesterol to brain cholesterol. These data will be invaluable in efforts to understand how plasma cholesterol levels may affect β -amyloid deposition in the brain. Additionally, several questions need to be answered to elucidate the molecular events underlying cholesterol's connection to APP processing. Does cholesterol interact directly with APP and/or the secretases? Does cholesterol require other unknown protein(s) to regulate APP processing? Finally, do cholesterol–A β interactions in plasma influence the overall accumulation of A β in the CNS? The coming years will surely witness new and exciting discoveries regarding the role of cholesterol in AD pathogenesis, thereby enabling new strategies for the prevention and treatment of this devastating neurodegenerative disease.

Acknowledgments

The work described in this review was supported by grants from the Alzheimer's Association (L.P.), the National Institute on Aging (R.E.T.), the National Institute of Neurological Disorders and Stroke (D.M.K.) and the American Health Assistance Foundation (AHAf; to D.M.K.).

RECEIVED 29 JANUARY; ACCEPTED 27 FEBRUARY 2003

1. Tanzi, R.E. & Bertram, L. New frontiers in Alzheimer's disease genetics. *Neuron* **32**, 181–184 (2001).
2. Thinakaran, G. The role of presenilins in Alzheimer's disease. *J. Clin. Invest.* **104**, 1321–1327 (1999).
3. Blacker, D. *et al.* Results of a high-resolution genome screen of 437 Alzheimer's Disease families. *Hum. Mol. Genet.* **12**, 23–32 (2003).
4. Selkoe, D.J. Translating cell biology into therapeutic advances in Alzheimer's disease. *Nature* **399**, 23–31 (1999).
5. Price, D.L., Tanzi, R.E., Borchelt, D.R., & Sisodia, S.S. Alzheimer's disease: genetic studies and transgenic models. *Annu. Rev. Genet.* **32**, 461–493 (1998).

6. Cao, X. & Sudhof, T.C. A transcriptionally active complex of APP with FE65 and histone acetyltransferase Tip60. *Science* **293**, 115–120 (2001).
7. Kimberly, W.T., Zheng, J.B., Guenette, S.Y. & Selkoe, D.J. The intracellular domain of the beta-amyloid precursor protein is stabilized by Fe65 and translocates to the nucleus in a Notch-like manner. *J. Biol. Chem.* **276**, 40288–40292 (2001).
8. Bales, K.R. *et al.* Lack of apolipoprotein E dramatically reduces amyloid β -peptide deposition. *Nat. Genet.* **17**, 263–264 (1997).
9. 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. USA* **96**, 15233–15238 (1999).
10. 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. USA* **97**, 2892–2897 (2000).
11. LaDu, M.J. *et al.* Isoform-specific binding of apolipoprotein E to β -amyloid. *J. Biol. Chem.* **269**, 23403–23406 (1994).
12. Naslund, J. *et al.* Characterization of stable complexes involving apolipoprotein E and the amyloid β peptide in Alzheimer's disease brain. *Neuron* **15**, 219–228 (1995).
13. Yang, D.S. *et al.* Apolipoprotein E promotes the binding and uptake of beta-amyloid into Chinese hamster ovary cells in an isoform-specific manner. *Neuroscience* **90**, 1217–1226 (1999).
14. Van Uden, E. *et al.* A protective role of the low density lipoprotein receptor-related protein against amyloid beta-protein toxicity. *J. Biol. Chem.* **275**, 30525–30530 (2000).
15. Chung, H., Brazil, M.L., Soe, T.T. & Maxfield, F.R. Uptake, degradation and release of fibrillar and soluble forms of Alzheimer's amyloid beta-peptide by microglial cells. *J. Biol. Chem.* **274**, 32301–32308 (1999).
16. Ulery, P.G. *et al.* Modulation of beta-amyloid precursor protein processing by the low density lipoprotein receptor-related protein (LRP). *J. Biol. Chem.* **275**, 7410–7415 (2000).
17. Herz, J. & Beffert, U. Apolipoprotein E receptors: linking brain development and Alzheimer's disease. *Nat. Rev. Neurosci.* **1**, 51–58 (2000).
18. Strittmatter, W.J. *et al.* Binding of human apolipoprotein E to synthetic amyloid β peptide: isoform-specific effects and implications for late-onset Alzheimer disease. *Proc. Natl. Acad. Sci. USA* **90**, 8098–8102 (1993).
19. Ma, J., Yee, A., Brewer, H.B. Jr., Das, S. & Potter, H. Amyloid-associated proteins alpha1-antichymotrypsin and apolipoprotein E promote assembly of Alzheimer β -protein into filaments. *Nature* **372**, 92–94 (1994).
20. Sanan, D.A. *et al.* Apolipoprotein E associates with β amyloid peptide of Alzheimer's disease to form novel monofibrils. Isoform apoE4 associates more efficiently than apoE3. *J. Clin. Invest.* **94**, 860–869 (1994).
21. 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).
22. Fagan, A.M. *et al.* Human and murine ApoE markedly alters A β metabolism before and after plaque formation in a mouse model of Alzheimer's disease. *Neurobiol. Dis.* **9**, 305–318 (2002).
23. Ehnholm, C., Lukka, M., Kuusi, T., Nikkila, E. & Utermann, G. Apolipoprotein E polymorphism in the Finnish population: gene frequencies and relation to lipoprotein concentrations. *J. Lipid Res.* **27**, 227–235 (1986).
24. Boerwinkle, E. *et al.* The use of measured genotype information in the analysis of quantitative phenotypes in man. II. The role of the apolipoprotein E polymorphism in determining levels, variability, and covariability of cholesterol, betalipoprotein, and triglycerides in a sample of unrelated individuals. *Am. J. Med. Genet.* **27**, 567–582 (1987).
25. Papassotiropoulos, A. *et al.* 24S-hydroxycholesterol in cerebrospinal fluid is elevated in early stages of dementia. *J. Psychiatr. Res.* **36**, 27–32 (2002).
26. Jarvik, G.P. *et al.* Interaction of apolipoprotein E genotype, total cholesterol level, and sex in prediction of Alzheimer disease in a case-control study. *Neurology* **45**, 1092–1096 (1995).
27. Kuo, Y.M. *et al.* Elevated low-density lipoprotein in Alzheimer's disease correlates with brain A β 1–42 levels. *Biochem. Biophys. Res. Commun.* **252**, 711–715 (1998).
28. Fernandes, M.A. *et al.* Effects of apolipoprotein E genotype on blood lipid composition and membrane platelet fluidity in Alzheimer's disease. *Biochim. Biophys. Acta.* **1454**, 89–96 (1999).
29. Knebl, J. *et al.* Plasma lipids and cholesterol esterification in Alzheimer's disease. *Mech. Ageing Dev.* **73**, 69–77 (1994).
30. Hofman, A. *et al.* Atherosclerosis, apolipoprotein E, and prevalence of dementia and Alzheimer's disease in the Rotterdam study. *Lancet* **349**, 151–154 (1997).
31. Mori, T. *et al.* Cholesterol accumulates in senile plaques of Alzheimer disease patients and in transgenic APP(SW) mice. *J. Neuropathol. Exp. Neurol.* **60**, 778–785 (2001).
32. Jick, H., Zornberg, G.L., Jick, S.S., Seshadri, S. & Drachman, D.A. Statins and the risk of dementia. *Lancet* **356**, 1627–1631 (2000).
33. Wolozin, B. & Behl, C. Mechanisms of neurodegenerative disorders: protein aggregates. *Arch. Neurol.* **57**, 793–796 (2000).
34. Yaffe, K., Barrett-Connor, E., Lin, F. & Grady, D. Serum lipoprotein levels, statin use, and cognitive function in older women. *Arch. Neurol.* **59**, 378–384 (2002).
35. Rockwood, K. *et al.* Use of lipid-lowering agents, indication bias and the risk

- of dementia in community-dwelling elderly people. *Arch. Neurol.* **59**, 223–227 (2002).
36. Simons, M. *et al.* Treatment with simvastatin in normocholesterolemic patients with Alzheimer's disease: a 26-week randomized, placebo-controlled, double-blind trial. *Ann. Neurol.* **52**, 346–350 (2002).
 37. Shepherd, J. *et al.* Pravastatin in elderly individuals at risk of vascular disease (PROSPER): a randomised controlled trial. *Lancet* **360**, 1623–1630 (2002).
 38. Petanceska, S., Papolla, M. & Refolo, L. Modulation of Alzheimer's amyloidosis by statins: mechanism of action. *Curr. Med. Chem. Immun. Endocrinol. Metab. Agents* **3**, 233–243 (2003).
 39. Corsini, A. Fluvastatin: effects beyond cholesterol lowering. *J. Cardiovasc. Pharmacol. Ther.* **5**, 161–175 (2000).
 40. Sparks, D.L. *et al.* Induction of Alzheimer-like beta-amyloid immunoreactivity in the brains of rabbits with dietary cholesterol. *Exp. Neurol.* **126**, 88–94 (1994).
 41. Sparks, D.L., Kuo, Y.M., Roher, A., Martin, T. & Lukas, R.J. Alterations of Alzheimer's disease in the cholesterol-fed rabbit, including vascular inflammation. Preliminary observations. *Ann. NY Acad. Sci.* **903**, 335–344 (2000).
 42. Refolo, L.M. *et al.* Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model. *Neurobiol. Dis.* **7**, 321–331 (2000).
 43. Refolo, L.M. *et al.* A cholesterol-lowering drug reduces beta-amyloid pathology in a transgenic mouse model of Alzheimer's disease. *Neurobiol. Dis.* **8**, 890–899 (2001).
 44. Howland, D.S. *et al.* Modulation of secreted β -amyloid precursor protein and amyloid β -peptide in brain by cholesterol. *J. Biol. Chem.* **273**, 16576–16582 (1998).
 45. Fassbender, K. *et al.* Simvastatin strongly reduces levels of Alzheimer's disease beta-amyloid peptides A β 42 and A β 40 *in vitro* and *in vivo*. *Proc. Natl. Acad. Sci. USA* **98**, 5856–5861 (2001).
 46. Refolo, L.M., Wittenberg, I.S., Friedrich V.L. Jr. & Robakis, N.K. The Alzheimer amyloid precursor is associated with the detergent-insoluble cytoskeleton. *J. Neurosci.* **11**, 3888–3897 (1991).
 47. Lee, S.J. *et al.* A detergent-insoluble membrane compartment contains A β *in vivo*. *Nat. Med.* **4**, 730–734 (1998).
 48. Ehehalt, R., Keller, P., Haass, C., Thiele, C. & Simons, K. Amyloidogenic processing of the Alzheimer beta-amyloid precursor protein depends on lipid rafts. *J. Cell Biol.* **160**, 113–123 (2003).
 49. Choo-Smith, L.P. & Surewicz, W.K. The interaction between Alzheimer amyloid beta(1-40) peptide and ganglioside GM1-containing membranes. *FEBS Lett.* **402**, 95–98 (1997).
 50. Yanagisawa, K., McLaurin, J., Michikawa, M., Chakrabarty, A. & Ihara, Y. Amyloid beta-protein (A β) associated with lipid molecules: immunoreactivity distinct from that of soluble A β . *FEBS Lett.* **420**, 43–46 (1997).
 51. Yanagisawa, K., Odaka, A., Suzuki, N. & Ihara, Y. GM1 ganglioside-bound amyloid β -protein (A β): a possible form of preamyloid in Alzheimer's disease. *Nat. Med.* **1**, 1062–1066 (1995).
 52. Simons, M. *et al.* Cholesterol depletion inhibits the generation of β -amyloid in hippocampal neurons. *Proc. Natl. Acad. Sci. USA* **95**, 6460–6464 (1998).
 53. Frears, E.R., Stephens, D.J., Walters, C.E., Davies, H. & Austen, B.M. The role of cholesterol in the biosynthesis of β -amyloid. *Neuroreport* **10**, 1699–1705 (1999).
 54. Bodovitz, S. & Klein, W.L. Cholesterol modulates alpha-secretase cleavage of amyloid precursor protein. *J. Biol. Chem.* **271**, 4436–4440 (1996).
 55. Racchi, M. *et al.* Secretory processing of amyloid precursor protein is inhibited by increase in cellular cholesterol content. *Biochem. J.* **322**, 893–898 (1997).
 56. 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. USA* **98**, 5815–5820 (2001).
 57. Runz, H. *et al.* Inhibition of intracellular cholesterol transport alters presenilin localization and amyloid precursor protein processing in neuronal cells. *J. Neurosci.* **22**, 1679–1689 (2002).
 58. Fukumoto, H., Deng, A., Irizarry, M.C., Fitzgerald, M.L. & Rebeck, G.W. Induction of the cholesterol transporter ABCA1 in central nervous system cells by liver X receptor agonists increases secreted A β levels. *J. Biol. Chem.* **277**, 48508–48513 (2002).
 59. Puglielli, L. *et al.* Acyl-coenzyme A: cholesterol acyltransferase modulates the generation of the amyloid beta-peptide. *Nat. Cell Biol.* **3**, 905–912 (2001).
 60. Chang, T.Y. *et al.* Roles of acyl-coenzyme A: cholesterol acyltransferase-1 and -2. *Curr. Opin. Lipidol.* **12**, 289–296 (2001).
 61. Chang, T.Y., Chang, C.C., Lu, X. & Lin, S. Catalysis of ACAT may be completed within the plane of the membrane. A working hypothesis. *J. Lipid Res.* **42**, 1933–1938 (2001).
 62. Brown, M.S. & Goldstein, J.L. A proteolytic pathway that controls the cholesterol content of membranes, cells and blood. *Proc. Natl. Acad. Sci. USA* **96**, 11041–11048 (1999).
 63. Tschape, J.A. *et al.* The neurodegeneration mutant lochrifg interferes with cholesterol homeostasis and A β processing. *EMBO J.* **21**, 6367–6376 (2002).
 64. Dietschy, J.M. & Turley, S.D. Cholesterol metabolism in the brain. *Curr. Opin. Lipidol.* **12**, 105–112 (2001).
 65. Snipes, G.J. & Suter, U. Cholesterol and myelin. in *Subcellular Biochemistry. Cholesterol: its Functions and Metabolism in Biology and Medicine* (ed. Bittman, R.) 173–204 (Plenum Press, New York, 1997).
 66. Accad, M. *et al.* Massive xanthomatosis and altered composition of atherosclerotic lesions in hyperlipidemic mice lacking acyl CoA: cholesterol acyltransferase 1. *J. Clin. Invest.* **105**, 711–719 (2000).
 67. Haley, R.W. & Dietschy, J.M. Is there a connection between the concentration of cholesterol circulating in plasma and the rate of neuritic plaque formation in Alzheimer disease? *Arch. Neurol.* **57**, 1410–1412 (2000).
 68. Salen, G. & Grundy, S.M. The metabolism of cholestanol, cholesterol and bile acids in cerebrotendinous xanthomatosis. *J. Clin. Invest.* **52**, 2822–2835 (1973).
 69. Salen, G. *et al.* Increased concentrations of cholestanol and apolipoprotein B in the cerebrospinal fluid of patients with cerebrotendinous xanthomatosis. Effect of chenodeoxycholic acid. *N. Engl. J. Med.* **316**, 1233–1238 (1987).
 70. Bjorkem, I. & Boberg, K.M. Inborn errors in bile acid biosynthesis and storage of sterols other than cholesterol. in *The Metabolic and Molecular Bases of Inherited Disease* (eds. Scriver, C.R., Beaudet, A.L., Sly, W.S. & Valle, D.) 2073–2099 (McGraw-Hill, New York, 1995).