Drugs of the Future 2002, 27(9): 863-868

CCC: 0377-8282/2002

Review Article

Is ACAT a novel pharmacological target for the treatment of Alzheimer's disease?

Luigi Puglielli

Genetics and Aging Research Unit, Center for Aging, Genetics and Neurodegeneration (CAGN), Massachusetts General Hospital and Harvard Medical School, Bldg. 114, 16th Street (C3009), Charlestown, MA 02129, USA

CONTENTS

Abstract

Several independent epidemiological studies have suggested a role for cholesterol in the pathogenesis of Alzheimer's disease (AD). Consistent with this role, elevated cholesterol levels have been reported to increase the biogenesis/deposition of amyloid β-peptide (Aβ) in the brains of AD transgenic mouse models. Both cellular and biochemical studies have now confirmed the potential role of cholesterol metabolism and distribution in the regulation of the very first step of AD pathogenesis: the biogenesis of Aβ. Although the molecular mechanisms involved in the sterol-dependent regulation of Aβ generation have not yet been completely revealed, recent reports seem to implicate AcylCoA:cholesterol acyltransferase (ACAT), a key enzyme in the regulation of intracellular cholesterol distribution.

Introduction

Alzheimer's disease (AD) represents the most common cause of dementia in the Western world and affects as many as 15 million individuals worldwide. It is characterized by progressive memory deficits, cognitive impairments and personality changes accompanied by diffuse structural abnormalities in the brain. The symptoms of the disease include memory loss, confusion, impaired judgment, personality changes, disorientation and loss of language skills.

Based on the onset of the symptoms, AD is normally divided into 2 groups: early-onset (< 60 years) and late-onset (> 60 years). Early-onset AD (also called familial AD) accounts for approximately 5% of all AD cases and has so far been linked to mutations in the genes for the amyloid precursor protein (APP), presenilin 1 and presenilin 2 (1). Late-onset AD accounts for the remaining 95% of all AD cases and has been associated with genetic polymorphisms that appear to operate as risk factors and/or genetic modifiers (2).

Late-onset AD is a complex and genetically heterogeneous disease and, together with other common disorders (*e.g.*, cardiovascular disease, diabetes and cancer), is one of the most common age-related diseases. Because of the shift in life expectancy we are experiencing, it is estimated that in 2050 about 25% of the population in the Western world will be over 65 years of age and one-third of them will be affected by AD.

The pathological and histological hallmarks of AD include extracellular protein deposits termed amyloid (or senile) plaques, neurofibrillary tangles and amyloid angiopathy accompanied by diffuse loss of neurons and synapses in the neocortex, hippocampus and other subcortical regions of the brain. Amyloid plaques represent the single most important pathological lesion. When found in sufficient numbers in limbic and association cortices, they alone allow a definitive postmortem diagnosis of AD. The dominant component of the plaque core is the amyloid beta (Aβ) peptide organized in fibrils of approximately 7-10 nm intermixed with nonfibrillar forms of this peptide.

The common pathogenic event that occurs in early- and late-onset AD is the abnormal accumulation of Aβ in the form of amyloid plaques. In the case of familial AD, the accumulation is mostly the result of increased production of a specific 42-amino acids isoform of Aβ ($A\beta_{42}$) that accelerates the aggregation and accumulation of total Aβ into amyloid fibrils. The only exceptions are trisomy 21 (Down's syndrome), where a third copy of the APP gene leads to an increased production of Aβ, and the APP "Swedish" mutation, which elevates total Aβ levels. In contrast to familial AD, the exact mechanisms that lead to the accumulation of Aβ in late-onset AD are still not completely known. The old dogma claiming a defect in Aβ clearance rather than production seems to

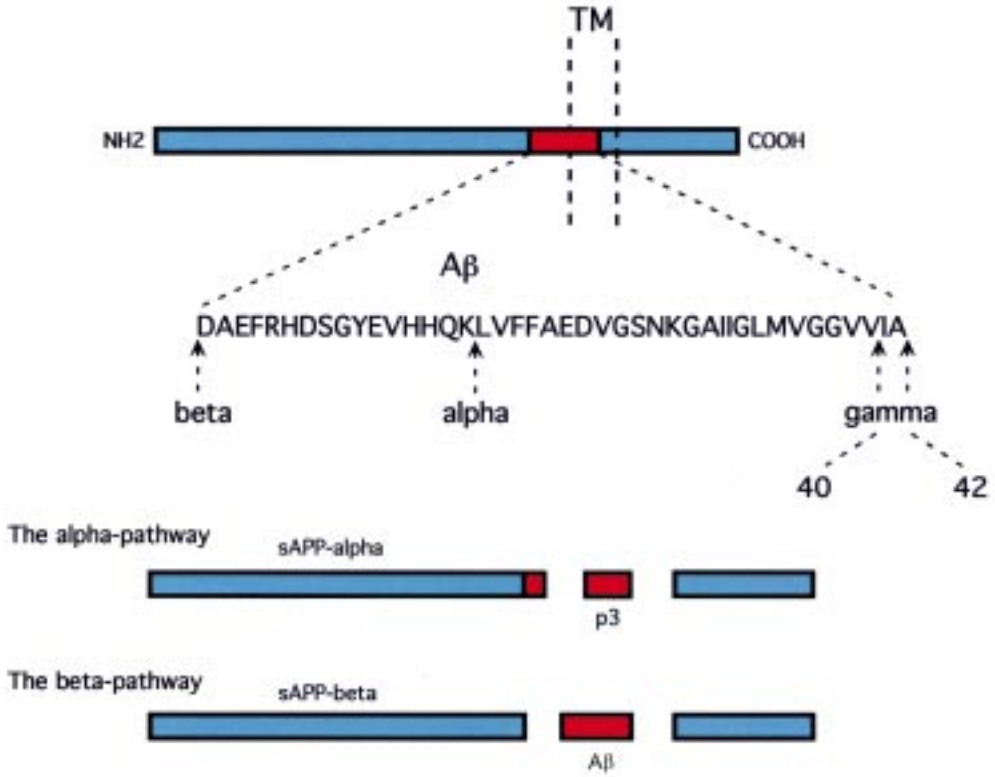

Fig. 1. Processing of the amyloid precursor protein (APP). The amyloid β protein (Aβ; in red) is generated by the sequential cleavage of APP along the beta-pathway. The alpha-pathway cleaves APP in the middle of the Aβ sequence and generates a small fragment (p3) that does not aggregate in amyloid plaques. The entire sequence of Aβ with the sites where alpha, beta and gamma cleavages occur is shown. TM indicates the transmembrane domain of APP.

be highly challenged by new biochemical studies. Additional information on the mechanisms involved in the abnormal accumulation of Aβ in early- and late-onset AD can be found elsewhere (1, 3, 4).

The biology of Alzheimer's disease

The purification of Aβ from amyloid deposits of AD patients paved the way for the cloning of the gene that encodes for APP (5). APP consists of 695-770 amino acid residues, with APP_{695}, APP_{751} and APP_{770} the most common forms expressed in the brain. They all originate from alternatively spliced mRNAs transcribed from a single gene.

APP is a type I glycoprotein with its amino terminus on the lumenal/extracellular surface, a single approximately 23-residue transmembrane domain and a short cytoplasmic tail (Fig. 1). Most of APP is cleaved at the alpha position, between amino acids 16 and 17 of the Aβ region, precluding the generation of Aβ, while producing a soluble, extracellular large NH_2-ectodomain (sAPP-alpha). The remaining C-terminal fragment can then be cleaved at the gamma position, in the short transmembrane domain producing a small 3-kDa Aβ fragment, which does not aggregate in amyloid plaques (Fig. 1, the alpha pathway). Full-length Aβ is produced by the beta pathway, where APP is first cleaved at the N-terminus of Aβ (beta-cleavage) and then in the transmembrane domain (gamma-cleavage). The proteases that cleave APP at alpha, beta and gamma position are called alpha-, beta- and gamma-secretases. Beta-secretase has recently been identified and renamed BACE (Beta-site APP Cleaving Enzyme). The identity of the gamma-secretase, possible presinilins themselves, is still under intense investigation (6, 7).

The majority of APP is normally cleaved along the alpha, rather than beta, pathway. The gamma-secretase usually cleaves APP either at position 40 or 42 of the Aβ region generating $Aβ_{40}$ and $Aβ_{42}$, respectively (Fig. 1) (3, 4). Normally, about 90% of secreted Aβ is 40 amino acids long and only about 10% is 42 amino acids long. Even if less prevalent, $Aβ_{42}$, with its 2 additional hydrophobic residues, aggregates far more rapidly into amyloid fibrils and is more toxic (1, 3, 4).

Cholesterol and AD

The past few years have seen the emergence of numerous epidemiological data suggesting that cholesterol levels may constitute a novel independent risk factor

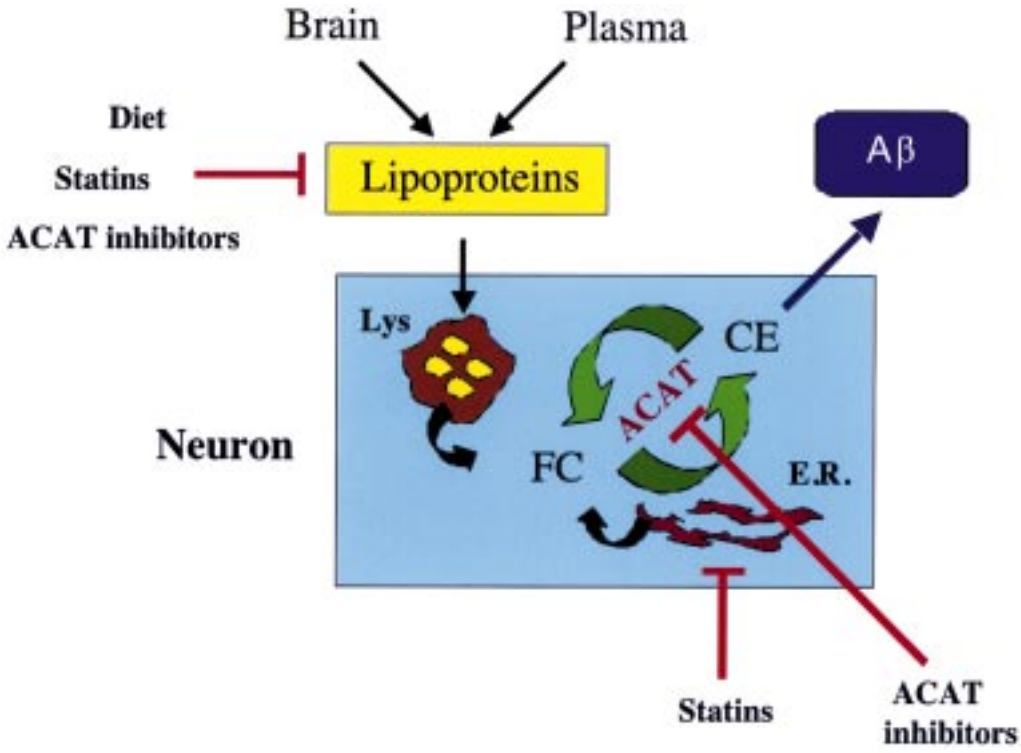

Fig. 2. Schematic view of cholesterol-mediated regulation of Aβ biogenesis and possible therapeutic targets. Neurons obtain cholesterol in the form of free cholesterol (FC), from *de novo* biosynthesis in the endoplasmic reticulum (ER) or receptor-mediated internalization of extracellular lipoproteins. Lipoproteins are mostly produced in the central nervous system (CNS) by astrocytes, but also transported from the plasma across the blood brain barrier. Lipoproteins undergo enzymatic digestion in the lysosomal compartment (Lys). Both newly synthesized and preformed cholesterol are substrate for the acyl-coenzyme A:cholesterol acyltransferase (ACAT), which generates cholesterol ester (CE). The equilibrium between FC and CE regulates the biogenesis of Aβ. Statins act at different levels: they reduce the secretion of plasma and CNS lipoproteins, and the biosynthesis on FC in neurons. These combined effects determine a decrease in the substrate available for the generation of CE. In contrast to statins, ACAT inhibitors mostly inhibit the last biochemical step in the CE biosynthetic pathway. In addition, they also contribute to the reduction of lipoprotein-derived cholesterol by acting at the intestinal and hepatic level (see Fig. 3). Hypocholesterolemic diets may also contribute to the prevention of AD by reducing the levels of lipoprotein-derived cholesterol.

for late-onset AD. This conclusion is mostly, but not only, based on the following 3 observations. First, individuals with elevated levels of plasma cholesterol have an increased susceptibility to AD (8, 9). Second, AD patients have increased levels of total serum and low-density lipoprotein (LDL) cholesterol when compared to age-matched controls (8, 10, 11). Third, AD patients have reduced levels of ApoA/high-density lipoprotein (HDL) in the plasma (11-13). This metabolic profile (hypercholesterolemia with high LDL-cholesterol and low HDL-cholesterol) is commonly found in patients with atherosclerosis. Interestingly, the presence of atherosclerosis, intimately related to hypercholesterolemia, has also been shown to correlate with an increased risk of AD, with the higher levels of risk being associated with more marked degrees of atherosclerosis (14).

The AD-cholesterol link has been further confirmed by several independent studies performed either with animal or cellular models of AD. It is worth noting that the animal studies have also broken the old dogma that plasma cholesterol cannot affect cholesterol metabolism in the brain. In fact, almost in perfect agreement with the epidemiological studies, Refolo *et al.* (15) and Howland *et al.* (16) have shown that a high fat/high cholesterol diet is able to increase cholesterol levels in both the plasma and the central nervous system (CNS) of transgenic mice. Refolo and colleagues also found that this effect is accompanied by a significant increase in Aβ levels in the brain, indicating that cholesterol levels can regulate Aβ generation *in vivo*. Neuropathological analysis showed that the hypercholesterolemic diet also increased the deposition of amyloid plaques. The effect of hypercholesterolemia on cholesterol metabolism and Aβ biogenesis in the brain could be reversed by the use of lipid-lowering drugs, including statins (17, 18).

These results have led to the concept that dietary or pharmacological modulation of cholesterol metabolism in the plasma and the brain may constitute an early strategy for the prevention of AD (Fig. 2). Indeed, two recent reports from Jick *et al.* (19) and Wolozin *et al.* (20) have

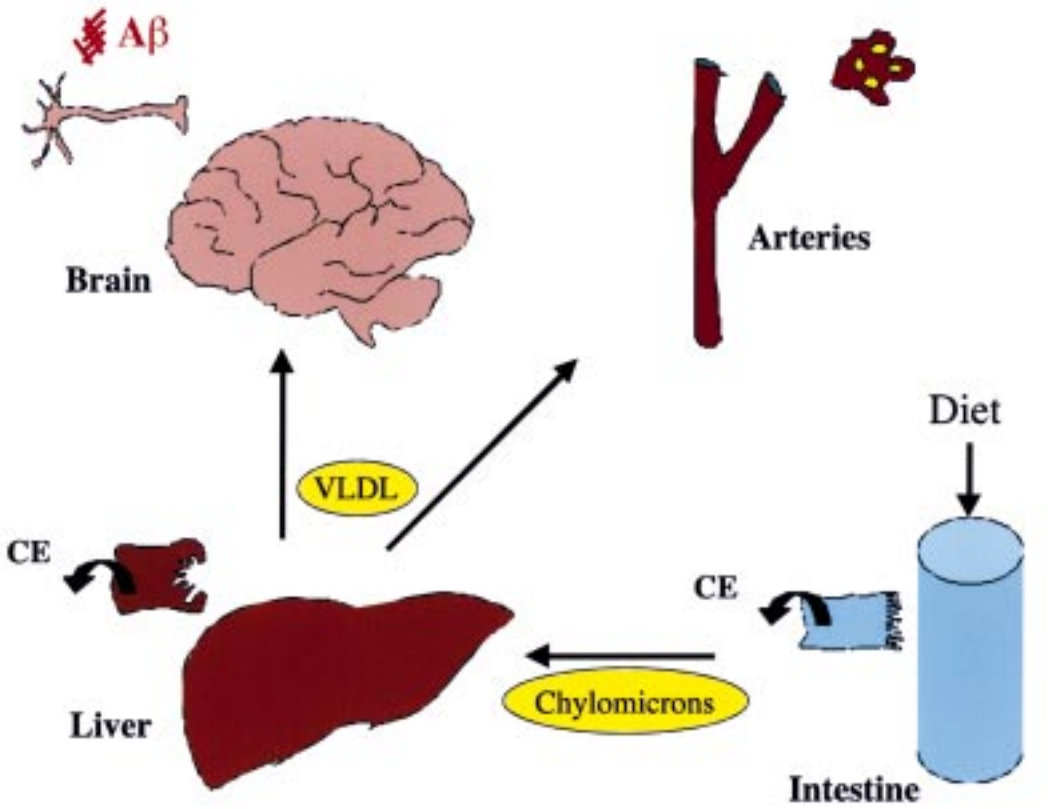

Fig. 3. Possible therapeutic targets of ACAT inhibitors. ACAT inhibitors reduce the secretion of chylomicrons from enterocytes, thereby affecting cholesterol absorption from the intestine. They also inhibit the generation and secretion of very low density lipoproteins (VLDL) from hepatocytes, thereby reducing the levels of lipoprotein-cholesterol circulating in the plasma. This would lead to a reduction in the input of plasma-derived cholesterol in the CNS. ACAT inhibitors can potentially block the formation of amyloid plaques in the brain preventing Alzheimer's disease, and of atherosclerotic plaques in the arteries preventing atherosclerosis.

shown that the use of statins to treat hypercholesterolemia also protects against AD. Despite the preliminary nature of the conclusions from these studies, they readily corroborate preexisting epidemiological and animal data. Results from ongoing clinical trials in the U.S. and Europe will help us understand the real potential of cholesterol-lowering agents in the treatment of AD.

ACAT: molecular link between AD and cholesterol

Mammalian cells obtain cholesterol in the form of free cholesterol from both *ex novo* biosynthesis and receptor-mediated internalization of plasma lipoproteins. This pool of free cholesterol is then stored in the form of cholesterol ester in cytoplasmic deposits called lipid droplets (Fig. 2). The pools of free cholesterol and cholesterol ester are in dynamic equilibrium with each other (21), and this equilibrium is tightly controlled by acyl-coenzyme A:cholesterol acyltransferase (ACAT), an endoplasmic reticulum resident enzyme that catalyzes the formation of cholesterol ester from cholesterol and long-chain fatty acids (22) (Fig. 2).

We have recently shown through genetic, biochemical and metabolic approaches that intracellular cholesterol distribution rather than total cholesterol levels regulates APP processing and Aβ generation (23). Our results indicate that ACAT modulates the generation of Aβ through the tight control of the equilibrium between free cholesterol and cholesterol ester. A selective increase in colesterol ester is sufficient to upregulate the generation of Aβ. This probably explains at the cellular level why the increase in cholesterol input in the brain upregulates Aβ generation and accumulation in animal models. We also showed that ACAT competitive inhibitors reduce both cholesterol ester and Aβ biosynthesis in a dose-dependent manner. This effect seems to require a net shift in cholesterol distribution from the pool of cholesterol ester to that of free cholesterol. Finally, ACAT activity was able to regulate APP processing and Aβ generation in several cell lines and in primary neurons. Runz *et al.* (24) recently confirmed our results, showing that interruption of cholesterol delivery to ACAT reduces Aβ generation. In conclusion, the above results have identified ACAT as a novel target for the pharmacological treatment of AD.

ACAT is a central enzyme in the regulation of cholesterol homeostasis and distribution in every single tissue of the body. In the small intestine, it regulates the secretion of chylomicrons into the lymphatic system, thereby controlling the rate of cholesterol absorption from the diet (Fig. 3). In the liver, ACAT controls the editing of the apolipoprotein B, thereby controlling the assembling and secretion of very low density lipoprotein (VLDL) into the blood (Fig. 3). Because of its role in the regulation of cholesterol metabolism, ACAT has been an important pharmacological target for the prevention and treatment of hypercholesterolemia. Indeed, several studies in both humans and laboratory animals have shown that competitive and noncompetitive inhibitors of ACAT activity reduce cholesterol levels in the blood by about 20% (22). In addition to its role in the intestine and liver, ACAT also plays a pivotal role in the accumulation of sterols in macrophages and smooth muscles cells in the wall of the arteries, thereby regulating the abnormal generation of foam cells in atherosclerotic plaques (Fig. 3) (22).

ACAT as a target for the prevention of AD

Even if early biochemical studies in laboratory animals had suggested that the brain is not able to generate cholesterol ester, the past few years have seen the emergence of new and strong evidence that seem to indicate the opposite. Indeed, molecular genetics has shown that mammals, including humans, express 2 different ACAT forms: ACAT-1 and ACAT-2 (25-29). While ACAT-2 is selectively expressed in the liver and intestine, ACAT-1 is almost uniformly distributed among several tissues, including the brain (27-29). In addition, biochemical and genetic studies indicate that the brain can regulate ACAT activity in response to changes in cholesterol biosynthesis and input (30, 31). The obvious question that remains to be addressed is: if ACAT is present and active in the brain, why is cholesterol ester not easily detected in the CNS? In fact, the major opposition to a possible involvement of ACAT in the pathogenesis of AD comes from the fact that enzymatic analysis of cholesterol distribution in the brain detects very small levels of cholesterol ester. However, these results are biased by the exceptionally high levels of free cholesterol contained in the CNS that are almost completely sequestered in the membranes of myelin (31). Indeed, analysis of cholesterol distribution in neuronal cell lines (human neuroglioma and neuroblastoma) and in primary neurons indicates that cholesterol ester constitutes about 30% of the entire pool of cellular cholesterol (23), strongly suggesting that neurons behave very similarly to peripheral cells.

ACAT is required for the biosynthesis of cholesterol ester which is then used to package chylomicrons in enterocytes and VLDL in hepatocytes (22). Inhibition of ACAT activity at these levels blocks cholesterol absorption from the diet and VLDL secretion into the plasma. These effects explain the reduction in cholesterolemia observed after treatment with ACAT inhibitors. In addition, ACAT inhibitors can potentially block cholesterol incorporation into lipid droplets, preventing the formation of foam cells in atherosclerotic plaques. The involvement of ACAT in the biogenesis of Aβ is another benefit associated with the use of ACAT inhibitors. The fact that ACAT inhibitors display different inhibitory activities for ACAT-1 and ACAT-2 (32) may even allow us to specifically target the brain *versus* the intestine/liver, or vice versa, according to different needs of the patients. Even though the above evidence supports the possible involvement of ACAT in the pathogenesis of AD and ACAT is proposed as a novel pharmacological target for AD treatment, the final answer will only come from animal studies. The coming years will witness new exciting discoveries that may help us prevent the most prevalent form of neurodegenerative disease.

References

1. Tanzi, R.E. *A genetic dichotomy model for the inheritance of Alzheimer's disease and common age-related disorders.* J Clin Invest 1999, 104: 1175-9.

2. Tanzi, R.E., Bertram, L. *New frontiers in Alzheimer's disease genetics.* Neuron 2001, 32: 181-4.

3. Selkoe, D.J. *Translating cell biology into therapeutic advances in Alzheimer's disease.* Nature 1999, 399: A23-31.

4. De Strooper, B., Annaert, W. *Proteolytic processing and cell biological functions of the amyloid precursor protein.* J Cell Sci 2000, 113: 1857-70.

5. Kang, J., Lemaire, H.G., Unterbeck, A. et al. *The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor.* Nature 1987, 325: 733-6.

6. Haass, C., De Strooper, B. *The presenilins in Alzheimer's disease-proteolysis holds the key.* Science 1999, 286: 916-9.

7. Wolfe, M.S., De los Angeles, J., Miller, D.D. et al. *Are presenilins intramembrane-cleaving proteases? Implications for the molecular mechanism of Alzheimer's disease.* Biochemistry 1999, 38: 11223-30.

8. Notkola, I.L., Sulkava, R., Pekkanen, J. et al. *Serum total cholesterol, apolipoprotein E ε 4 allele, and Alzheimer's disease.* Neuroepidemiology 1998, 17: 14-20.

9. Koudinov, A.R., Berezov, T.T., Koudinova, N.V. *Alzheimer's amyloid β and lipid metabolism: A missing link?* Faseb J 1998, 12: 1097-9.

10. Jarvik, G.P., Wijsman, E.M., Kukull, W.A. et al. *Interaction of apolipoprotein E genotype, total cholesterol level, and sex in prediction of Alzheimer disease in a case-control study.* Neurology 1995, 45: 1092-6.

11. Kuo, Y.M., Emmerling, M.R., Bisgaier, C.L. et al. *Elevated low-density lipoprotein in Alzheimer's disease correlates with brain Aβ 1-42 levels.* Biochem Biophys Res Commun 1998, 252: 711-5.

12. Fernandes, M.A., Proenca, M.T., Nogueira, A.J. et al. *Effects of apolipoprotein E genotype on blood lipid composition and membrane platelet fluidity in Alzheimer's disease.* Biochim Biophys Acta 1999, 1454: 89-96.

13. Merched, A., Xia, Y., Visvikis, S. et al. *Decreased high-density lipoprotein cholesterol and serum apolipoprotein AI concen-*

trations are highly correlated with the severity of Alzheimer's disease. Neurobiol Aging 2000, 21: 27-30.

14. Hofman, A., Ott, A., Breteler, M.M. et al. *Atherosclerosis, apolipoprotein E, and prevalence of dementia and Alzheimer's disease in the Rotterdam Study.* Lancet 1997, 349: 151-4.

15. Refolo, L.M., Pappolla, M.A., Malester, B. et al. *Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model.* Neurobiol Dis 2000, 7: 321-31.

16. Howland, D.S., Trusko, S.P., Savage, M.J. et al. *Modulation of secreted β-amyloid precursor protein and amyloid β-peptide in brain by cholesterol.* J Biol Chem 1998, 273: 16576-82.

17. Refolo, L.M., Pappolla, M.A., LaFrancois, J. et al. *A cholesterol-lowering drug reduces β-amyloid pathology in a transgenic mouse model of Alzheimer's disease.* Neurobiol Dis 2001, 8: 890-9.

18. Fassbender, K., Simons, M., Bergmann, C. et al. *Simvastatin strongly reduces levels of Alzheimer's disease β-amyloid peptides Aβ 42 and Aβ 40 in vitro and in vivo.* Proc Natl Acad Sci USA 2001, 98: 5856-61.

19. Jick, H., Zornberg, G.L., Jick, S.S. et al. *Statins and the risk of dementia.* Lancet 2000, 356: 1627-31.

20. Wolozin, B., Behl, C. *Mechanisms of neurodegenerative disorders. Part 1: protein aggregates.* Arch Neurol 2000, 57: 793-6.

21. Brown, M.S., Ho, Y.K., Goldstein, J.L. *The cholesteryl ester cycle in macrophage foam cells. Continual hydrolysis and re-esterification of cytoplasmic cholesteryl esters.* J Biol Chem 1980, 255: 9344-52.

22. Chang, T.Y., Chang, C.C., Cheng, D. *Acyl-coenzyme A:cholesterol acyltransferase. Annu Rev Biochem* 1997, 66: 613-38.

23. Puglielli, L., Konopka, G., Pack-Chung, E. et al. *Acyl-coenzyme A:cholesterol acyltransferase modulates the generation of the amyloid beta-peptide.* Nat Cell Biol 2001, 3: 905-12.

24. Runz, H., Rietdorf, J., Tomic, I. et al. *Inhibition of intracellular cholesterol transport alters presenilin localization and amyloid precursor protein processing in neuronal cells.* J Neurosci 2002, 22: 1679-89.

25. Chang, C.C., Huh, H.Y., Cadigan, K.M. et al. *Molecular cloning and functional expression of human acyl-coenzyme A:cholesterol acyltransferase cDNA in mutant Chinese hamster ovary cells.* J Biol Chem 1993, 268: 20747-55.

26. Meiner, V.L., Cases, S., Myers, H.M. et al. *Disruption of the acyl-CoA:cholesterol acyltransferase gene in mice: Evidence suggesting multiple cholesterol esterification enzymes in mammals.* Proc Natl Acad Sci USA 1996, 93: 14041-6.

27. Cases, S., Novak, S., Zheng, Y.W. et al. *ACAT-2, a second mammalian acyl-CoA:cholesterol acyltransferase. Its cloning, expression, and characterization.* J Biol Chem 1998, 273: 26755-64.

28. Oelkers, P., Behari, A., Cromley, D. et al. *Characterization of two human genes encoding acyl coenzyme A:cholesterol acyltransferase-related enzymes.* J Biol Chem 1998, 273: 26765-71.

29. Anderson, R.A., Joyce, C., Davis, M. et al. *Identification of a form of acyl-CoA:cholesterol acyltransferase specific to liver and intestine in nonhuman primates.* J Biol Chem 1998, 273: 26747-54.

30. Accad, M., Smith, S.J., Newland, D.L. et al. *Massive xanthomatosis and altered composition of atherosclerotic lesions in hyperlipidemic mice lacking acyl CoA:cholesterol acyltransferase 1.* J Clin Invest 2000, 105: 711-9.

31. Snipes, G.J., Suter, U. *Cholesterol and myelin.* In: Subcellular Biochemistry. Cholesterol: Its Functions and Metabolism in Biology and Medicine. Bittman, R. (Ed.). Plenum Press: New York 1997, 173-204.

32. Chang, C.C., Sakashita, N., Ornvold, K. et al. *Immunological quantitation and localization of ACAT-1 and ACAT-2 in human liver and small intestine.* J Biol Chem 2000, 275: 28083-92.