Cholesterol and apolipoprotein E in Alzheimer's disease

Allison B. Reiss, MD

Abstract

Alzheimer's disease (AD) is the most common cause of dementia in North America and Europe. The incidence of the disease rises dramatically with age. AD is a complex multifactorial disorder that involves numerous susceptibility genes, but the exact pathogenesis and biochemical basis of AD is not well understood. Cholesterol is receiving a great deal of attention as a potentially crucial factor in the etiology of AD. Almost all cholesterol in the brain is synthesized in the brain. Cholesterol exits the brain through the blood-brain barrier (BBB) in the form of apolipoprotein E (ApoE) or by first being converted to a more polar compound, 24(S)-hydroxycholesterol, which is elevated in individuals with AD. The key event leading to AD appears to be the formation and aggregation in the brain of amyloid β (Aβ) peptide, a proteolytically derived product of amyloid precursor protein (APP). Cholesterol has been demonstrated to modulate processing of APP to Aβ. High levels of cholesterol are associated with increased risk of AD. Patients taking cholesterol-lowering statins have a lower prevalence of AD. ApoE, which transports cholesterol throughout the brain, exhibits an isoform-specific association with AD such that the E4 isoform, by unknown mechanisms, shifts the onset curve toward an earlier age.

Key words: Alzheimer's disease, amyloid β peptide, apolipoprotein E, cholesterol

Introduction

Alzheimer's disease (AD) is a progressive, irreversible brain disorder with no known cure. It causes a gradual decline in memory and language skills, mood changes, confusion, disorientation in time and space, and, eventually, loss of the ability to care for oneself. AD is the most common form of irreversible dementia.[1] Effective pharmacological treatment of AD is lacking despite extensive efforts to produce active therapy aimed at neuronal and cerebrovascular targets.[2] Although the cause or causes of AD are still unknown, the pathological features that characterize the disease are the deposition of 39-42 amino acid peptides termed amyloid β (Aβ) and the appearance of neurofibrillary tangles in the hippocampus and cerebral cortex in the brains of those with AD. The amyloid hypothesis postulates that AD owes to the aggregation and accumulation of Aβ in affected brain regions.[3] It is the endoproteolytic cleavages of amyloid precursor protein (APP), a large type I transmembrane protein, by β- and γ-secretases that results in the generation of Aβ peptides that are thought to be neurotoxic. A third enzyme, the α-secretase, cleaves APP within the Aβ region and thus prevents Aβ formation. Both α- and β-secretases directly compete for their substrate, APP.[4]

There is strong evidence that alterations in lipid metabolism play a role in the pathogenesis of AD.[5,6] The ApoE E4 allele, which exacerbates hypercholesterolemia, is a dose-dependent risk factor for the development of AD,[7] and cholesterol plays an intrinsic role in AD pathogenesis.[8] High serum total cholesterol may be an independent risk factor for AD. Thus, proteins and transporters involved in maintaining brain cholesterol homeostasis are likely participants in the development of AD. The targeting and appropriate manipulation of the activity/expression of brain cholesterol transport proteins could provide an approach to the stabilization, slowing, or prevention of AD.

Brain cholesterol balance and the blood-brain barrier

The brain is the organ richest in cholesterol.[9] The central nervous system (CNS) contains approximately one-fourth

Allison B. Reiss, MD, Vascular Biology Institute, Department of Medicine, Winthrop-University Hospital, Mineola, New York.

of all the unesterified cholesterol present in the body. Brain cholesterol turnover is very slow.[9,10] Cholesterol homeostasis in the brain is uniquely separate from the rest of the body owing to the blood-brain barrier (BBB), which regulates the interface between the circulating blood and the brain.[11] This barrier is formed by the capillary endothelial cells in the walls of the vessels that carry blood into the brain. The BBB strictly limits transport into the brain through tight junctions between cerebral endothelial cells. Brain capillary endothelial cells possess few pinocytic vesicles. Passage across the endothelial cell membranes is determined by solubility in the lipid bilayer or recognition by a transport molecule. The BBB effectively prevents the brain from competing for cholesterol derived from circulating plasma lipoproteins. Therefore, nearly all brain cholesterol is a product of local de novo synthesis.[12] Cholesterol balance in the brain is dependent on local synthesis and brain-specific elimination via the secretion of cholesterol in the form of the metabolite 24(S)-hydroxycholesterol, as well as through the lipoprotein carrier ApoE.

Cholesterol in Alzheimer's disease pathogenesis

There are epidemiologic and biochemical data linking cholesterol, APP processing, Aβ, and AD. There are human studies that support the hypothesis that high serum cholesterol level is an independent risk factor for the development of AD[13] and mild cognitive impairment.[14] In a population-based sample of 444 men aged 70 to 89 years, high serum cholesterol level was a significant predictor of the prevalence of AD after controlling for age and the presence of the apoE epsilon 4 allele.[13] Recent epidemiological studies show a strong reduction in the incidence of AD in patients treated with cholesterol-lowering statins [i.e., inhibitors of 3-hydroxy-3-methyl-glutaryl CoA (HMG-CoA) reductase].[15,16]

Elevated cholesterol levels increase Aβ in cellular and animal models of AD,[17,18] and drugs that inhibit cholesterol synthesis lower Aβ in these models.[19,20] Modulation of cholesterolemia in a transgenic mouse model of AD with a high-fat/high-cholesterol diet increases Aβ accumulation and accelerates the AD-related amyloid pathology.[21] The widely prescribed cholesterol-lowering medications simvastatin and lovastatin reduce intracellular and extracellular levels of Aβ42 and Aβ40 peptides in primary cultures of rat hippocampal neurons and mixed cortical neurons infected with Semliki Forest Virus encoding the human APP.[17] Guinea pigs treated for three weeks with high doses of simvastatin exhibit a strong and reversible reduction of cerebral Aβ42 and Aβ40 levels in the cerebrospinal fluid and brain homogenate.[17] In a cell culture model of APP overexpression, addition of cholesterol to the culture medium diverted APP metabolism away from the non-amyloidogenic pathway mediated by α-secretase, producing a decrease in soluble APP.[22] Excessive cholesterol feeding with a 2 percent cholesterol diet induces accumulation of intracellular immunolabeled Aβ in New Zealand white rabbits.[18] All these findings demonstrate that cholesterol can modulate the yield of Aβ, and this has focused attention on cholesterol-reducing drugs as a potential treatment of AD. The mechanism by which cholesterol influences APP processing is still unknown, however, and may be direct or indirect.

Statins and Alzheimer's disease

HMG-CoA reductase inhibitors (i.e., lipid-lowering drugs commonly referred to as statins) significantly reduce mortality for patients with coronary artery disease.[23] The prevalence of AD is also reduced among people taking drugs of this class.[15,16] Statins may act directly on the processing of APP by inhibiting the α- and the β-secretase pathways.[24] In one study of elderly nondemented subjects, however, treatment with statins in the therapeutic range failed to significantly alter cerebrospinal fluid (CSF) concentrations of Aβ despite a substantial effect on cholesterol turnover.[25] A number of properties other than lipid lowering may help to explain the impact of statins on AD. The clinical action of many cholesterol-lowering drugs is the result of pleiotropic effects rather than simply a reduction in cholesterol. The documented beneficial effects are antiatherosclerotic, anti-inflammatory, and antithrombotic. Statins have been shown to reduce the levels of proinflammatory cytokines and markers of acute phase response including C-reactive protein and serum amyloid A.[26] They both have antioxidant properties and can inhibit the generation of reactive oxygen species and blunt their destructive effects.[27] Statins may also influence ApoE levels. In cultured astrocytes and microglia, exposure to statins decreases cellular and/or secreted ApoE.[28] Statin treatment significantly lowers plasma levels of 24(S)-hydroxycholesterol, an oxysterol generated in the brain by the cholesterol 24(S)-hydroxylase enzyme.[29] The effect of statins on 27-hydroxycholesterol, the other oxysterol present in the brain, have not yet been evaluated.[30] Statins active in preventing AD do not have to cross the BBB to exert their effects. They can alter cholesterol distribution in the brain without directly affecting brain cholesterol synthesis.[31]

Much of our knowledge of statin effects on brain cholesterol comes from animal studies. In guinea pigs, high-dose treatment with simvastatin or pravastatin did not alter

total brain cholesterol levels. A high-cholesterol diet elevated serum cholesterol in these animals, but did not affect total brain cholesterol level. De novo cholesterol synthesis in the guinea pig brain is downregulated by statins, as indicated by lower absolute levels and cholesterol-related ratios of the cholesterol precursor lathosterol compared with controls.[32]

Cholesterol 24-hydroxylase and reverse cholesterol transport

When intracellular cholesterol levels are elevated, an efflux system responds to maintain cholesterol equilibrium. Cholesterol is a highly hydrophobic lipid that requires specialized transport via lipoproteins or conversion to a more polar form. Cerebral cholesterol elimination involves both mechanisms: one dependent on ApoE and the other on the enzyme cholesterol 24-hydroxylase, a cytochrome P-450 species denoted Cyp46.[33] ApoE, the most abundant apolipoprotein in the CNS, is involved in the transport of cholesterol to neurons and is found in high-density lipoprotein—like lipoproteins in the CSF.[12]

In the brain, cholesterol is converted to 24(S)-hydroxycholesterol by Cyp46. In humans, 24(S)-hydroxycholesterol is formed almost exclusively in the brain via mitochondrial hydroxylation of cholesterol.[10] This polar cholesterol derivative continuously crosses the BBB and is readily secreted into the circulation. 24(S)-Hydroxycholesterol is a member of a family of oxygenated derivatives of cholesterol, termed oxysterols, which function as transport forms of cholesterol. Oxysterols regulate export of cholesterol from the cell and are critical intracellular signal transduction molecules that exert tight control over cellular cholesterol trafficking. They modulate transcription of genes related to cholesterol metabolism, thus mediating a number of cholesterol-induced metabolic effects, and they participate in cell signaling as ligands for the nuclear receptors liver X receptor α and β.[34] 24(S)-hydroxycholesterol is a cholesterol catabolite that is ultimately converted into bile acids or excreted in bile in its sulfated and glucuronidated form.

In the early stages of AD, serum and CSF 24-hydroxycholesterol levels are elevated when compared to healthy subjects. This elevation can be attributed to conversion of cholesterol from damaged or dying neurons to 24(S)-hydroxycholesterol.[35] Because almost all 24(S)-hydroxycholesterol is derived from the CNS, circulating levels of 24(S)-hydroxycholesterol reflect brain cholesterol catabolism. This suggests the possibility that serum 24-hydroxycholesterol may have clinical utility as a marker for early AD.[36,37] In AD patients, severity of dementia is independently associated with a decrease in the ratio of plasma 24-hydroxycholesterol/cholesterol. This reduction in the ratio of plasma 24(S)-hydroxycholesterol/cholesterol may be attributed to a loss of cholesterol 24(S)-hydroxylase in the CNS.[36]

Polymorphisms in Cyp46 are associated with an increased load of Aβ in AD, providing further evidence that abnormalities in the metabolism of cholesterol might contribute to the pathophysiology of AD.[38] The association between polymorphisms in the Cyp46 gene and AD susceptibility is controversial. Papassotiropoulos observed that brain Aβ load in subjects with a Cyp46 polymorphism 151 bases upstream of exon 3 was significantly higher than in subjects not carrying the polymorphism.[39] CSF levels of Aβ and phosphorylated tau protein were also elevated. In two independent populations, this genetic polymorphism was associated with higher risk of late-onset sporadic AD. In contrast, a study by Desai[40] using a large American white case-control cohort failed to support the association between the same polymorphism studied by Papassotiropoulos in Europe[39] and AD among American white or African-American subjects. A second Cyp46 polymorphism was examined by Kolsch.[41] This polymorphism involved a C to T transition in intron 3, 43 base pairs upstream of exon 3 of Cyp46. Carriers of the C allele of this polymorphism were more frequent in AD patients compared to healthy controls, and carriers of the CC genotype had significantly higher 24-hydroxycholesterol-cholesterol ratios in CSF than carriers of the CT and TT genotypes.

Alzheimer's disease and apolipoprotein E

Human ApoE, a small plasma protein of 299 amino acid residues, is a component of several different types of lipoproteins and plays a central role in cholesterol uptake and transport.[42] In the general population, ApoE exists in three major isoforms—ApoE2, ApoE3, and ApoE4—each differing by cysteine and arginine at positions 112 and 158. ApoE3, the most abundant allele, contains cysteine and arginine at both positions. The ApoE2 variant, the least common form, contains a cysteine substitution within the binding region at amino acid 158, and ApoE4 contains arginine at sites 112 and 158.[43,44] ApoE is expressed throughout the brain and is produced mainly in astrocytes and microglia. ApoE is involved in the regulation of cholesterol transport in the CNS.[45] ApoE is localized in the senile plaques, congophilic angiopathy, and neurofibrillary tangles in the brains of patients with AD. This plaque formation seems to involve an interaction between ApoE and Aβ peptide. ApoE binds Aβ with high avidity. ApoE3 and ApoE4 may behave differently and differentially contribute to the risk of AD.

The ApoE4 allele (chromosomal locus 19q13) is the single most important genetic risk factor associated with

AD. This allele confers increased risk for sporadic as well as familial AD.[46,47] ApoE4 is a major risk factor for AD in persons of both sexes aged 40 to 90 years, and persons with the genotype ApoE4/ApoE4 have the highest risk of developing AD (odds ratio 14.9, 95 percent CI 10.8 to 20.6).[48] Epidemiological studies have demonstrated that inheritance of one or more ApoE4 alleles affects the age of onset and the degree of amyloidogenesis. Patients expressing the ApoE4 allele have an earlier age at onset and a greater amyloid burden.[46,49] In susceptible individuals, the age at onset of AD averages approximately five to 10 years earlier in the presence of one allele and 10 to 20 years earlier in the presence of two alleles.[48] It is estimated that a young asymptomatic person with no family history of AD carrying one ApoE4 allele has an approximate doubling of lifetime risk of developing AD, from 15 percent to 29 percent.[50,51] Furthermore, whereas the ApoE4 allele defines a greater risk, the presence of ApoE4 is neither necessary nor sufficient to predict the disorder before the onset of symptoms. Only 40 percent of all AD patients have the ApoE4 allele. At this time, ApoE genotyping is not recommended for use in predictive testing or for routine clinical diagnosis of AD.[52]

In humans, the presence of one or more ApoE2 alleles appears to be protective, by lowering AD risk and increasing age of onset. In a murine model, dosage of ApoE2 and E3 alleles appears to be protective against the effects of ApoE4. In a transgenic mouse model using mice that overexpress APP and express different levels of human ApoE3, ApoE3 provided a dose-dependent protective effect against Aβ deposition.[53]

Although the precise pathological roles of ApoE remain to be clarified, multiple actions of ApoE affect Aβ and may impact AD. ApoE promotes the polymerization of Aβ into amyloid filaments,[54,55] may reduce Aβ clearance,[56] and may participate in CNS Aβ metabolism.[57] In addition, ApoE may have direct neurotoxic effects.[58] ApoE4 facilitates the deposition of Aβ as amyloid. In AD, the number of ApoE4 alleles correlates positively with increased deposits of Aβ.[49]

Interestingly, the association of ApoE genotype to level of C-reactive protein, an acute phase reactant produced in response to proinflammatory cytokines, is paradoxical. ApoE4 alleles are associated with lower circulating levels of C-reactive protein. A 34 percent decrease in this protein is seen in persons with one or two ApoE4 alleles, as compared to those with no ApoE4 alleles.[59] This effect is likely not related to inflammation because fibrinogen concentration and white blood cell count, both recognized markers of systemic inflammation, are unaffected. It has been speculated that ApoE polymorphisms cause alterations in the metabolism of plasma lipoproteins that indirectly affect C-reactive protein.[60] Western blotting and immunohistochemical studies have detected an increase in C-reactive protein associated with senile plaques and neurofibrillary tangles in the AD brain, but the relationship of C-reactive protein in the brain to ApoE genotype has not been reported.[61]

The ApoE4 allele is not only associated with an increased risk of AD, but also confers an increased risk of cardiovascular disease.[62] Population studies have shown that individuals carrying the ApoE4 allele display elevated levels of plasma cholesterol and low-density lipoprotein, even with a single copy of the gene.[63] Carriers of ApoE4 absorb cholesterol more effectively than subjects with other ApoE genotypes. The relationship between peripheral cholesterol level and risk of developing AD in persons with the ApoE4 allele is unknown.

Conclusions

There is currently no cure for AD. However, progress is being made toward an understanding of the pathobiologic mechanisms that lead to the destructive plaques and tangles found in the AD brain. Evidence is accumulating that brain cholesterol is involved in the formation of amyloid plaques.

The presence of an ApoE4 allele or alleles is neither necessary nor sufficient to establish a diagnosis of AD. Most people with AD do not carry the ApoE4 gene, and many people who carry ApoE4 do not develop AD.[51] It is probable that combinations of genes are involved in this disease. An example of genes acting together to increase AD susceptibility is seen in the synergistic increase in risk of AD in persons who carry Cyp46 and ApoE polymorphisms. Those carrying Cyp46 and ApoE4 variants were almost 10 times more likely to develop AD than those with neither variation. They also had the highest brain levels of Aβ.[39]

Taking cholesterol-lowering drugs may benefit those with AD. New treatment approaches designed to manipulate cholesterol metabolism, transport, and distribution in the brain may hold promise. This is clearly a fertile area for future exploration.

Acknowledgment

This work was supported in part by a grant from the Neuroscience Education and Research Foundation.

References

1. Clark C, Karlawish J: Alzheimer disease: Current concepts and emerging diagnostic and therapeutic strategies. *Ann Intern Med*. 2003; 138: 400-410.
2. Knopman D: Pharmacotherapy for Alzheimer's disease: 2002. *Clinical Neuropharmacol*. 2003; 26: 93-101.
3. Hardy J, Selkoe D: The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science*. 2002; 297: 353-356.

4. De Strooper B, Annaert W: Proteolytic processing and cell biological functions of the amyloid precursor protein. *J Cell Sci*. 2000; 113:1857-1870.
5. Hartmann T: Cholesterol, A beta and Alzheimer's disease. *Trend Neurosci*. 2001; 24: S45-S48.
6. Launer L, White L, Petrovitch H, et al.: Cholesterol and neuropathologic markers of AD: A population-based autopsy study. *Neurology*. 2001; 57: 1447-1452.
7. Strittmatter W, Roses A: Apolipoprotein E and Alzheimer disease. *Proc Natl Acad Sci USA* 1995; 92: 4725-4727.
8. Puglielli L, Tanzi R, Kovacs D: Alzheimer's disease: The cholesterol connection. *Nat Neurosci*. 2003; 6: 345-351.
9. Dietschy JM, Turley SD: Cholesterol metabolism in the brain. *Curr Opin Lipidol*. 2001; 12: 105-112.
10. Björkhem I, Lütjohann D, Diczfalusy U, et al.: Cholesterol homeostasis in human brain: Turnover of 24S-hydroxycholesterol and evidence for a cerebral origin of most of this oxysterol in the circulation. *J Lipid Res*. 1998; 39: 1594-1600.
11. Ballabh P, Braun A, Nedergaard M: The blood-brain barrier. An overview: Structure, regulation, and clinical implications. *Neurobiol Dis*. 2004; 16: 1-13.
12. Bjorkhem I, Meaney S: Brain cholesterol: Long secret life behind a barrier. *Arterioscler Thromb Vasc Biol*. 2004; 24: 806-815.
13. Notkola I-L, Sulkava R, Pekkanen J, et al.: Serum total cholesterol, apolipoprotein E 4, and Alzheimer's disease. *Neuroepidemiology*. 1998; 17: 14-20.
14. Kivipelto M, Helkala E-L, Hänninen T, et al.: Midlife vascular risk factors and late-life mild cognitive impairment. A population-based study. *Neurology*. 2001; 56: 1683-1689.
15. Wolozin B, Kellman W, Ruosseau P, et al.: Decreased prevalence of Alzheimer disease associated with 3-hydroxy-3-methyglutaryl coenzyme A reductase inhibitors. *Arch Neurol*. 2000; 57: 1439-1443.
16. Jick H, Zornberg G, Jick S, et al.: Statins and the risk of dementia. *Lancet*. 2000; 356: 1627-1631.
17. Fassbender K, Simons M, Bergmann C, et al.: Simvastatin strongly reduces levels of Alzheimer's disease beta-amyloid peptides Abeta 42 and Abeta 40 in vitro and in vivo. *Proc Natl Acad Sci USA*. 2001; 98: 5856-5861.
18. Sparks D, Scheff S, Hunsaker J, et al.: Induction of Alzheimer-like b-amyloid immunoreactivity in the brains of rabbits with dietary cholesterol. *Exper Neurol*. 1994; 126: 88-94.
19. Simons M, Keller P, De Strooper B, et al.: Cholesterol depletion inhibits the generation of beta-amyloid in hippocampal neurons. *Proc Natl Acad Sci USA*. 1998; 95: 6460-6464.
20. Buxbaum J, Geoghagen N, Friedhoff L: Cholesterol depletion with physiological concentrations of a statin decreases the formation of the Alzheimer amyloid Abeta peptide. *J Alz Dis*. 2001; 3: 221-229.
21. Refolo L, Malester B, LaFrancois J, et al.: Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model. *Neurobiol Dis*. 2000; 7: 321-331.
22. Bodovitz S, Klein W: Cholesterol modulates alpha-secretase cleavage of amyloid precursor protein. *J Biol Chem*. 1996; 271: 4436-4440.
23. Fonarow G, Watson K: Effective strategies for long-term statin use. *Am J Cardiol*. 2003; 92(1A): 27i-34i.
24. Sjogren M, Gustafsson K, Syversen S, et al.: Treatment with simvastatin in patients with Alzheimer's disease lowers both alpha- and beta-cleaved amyloid precursor protein. *Dement Geriatr Cog Dis*. 2003; 16(1): 25-30.
25. Fassbender K, Stroik M, Bertsch T, et al.: Effects of statins on human cerebral cholesterol metabolism and secretion of Alzheimer amyloid peptide. *Neurology*. 2002; 59: 1257-1258.
26. Vaughan C, Gotto A, Basson C: The evolving role of statins in the management of atherosclerosis. *J Am Coll Cardiol*. 2000; 35: 1-10.
27. Stoll L, McCormick M, Denning G, et al.: Antioxidant effect of statins. *Drugs Today*. 2004; 40: 975-990.
28. Petanceska S, DeRosa S, Sharma A, et al.: Changes in apolipoprotein E expression in response to dietary and pharmacological modulation of cholesterol. *J Molec Neurosci*. 2003; 20: 395-406.
29. Vega G, Weiner M, Lipton A, et al.: Reduction in levels of 24S-hydroxycholesterol by statin treatment in patients with Alzheimer disease. *Arch Neurol*. 2003; 60: 510-515.
30. Brown J, Theisler C, Silberman S, et al.: Differential expression of cholesterol hydroxylases in Alzheimer's disease. *J Biol Chem*. 2004; 279: 34674-34681.
31. Kirsch C, Eckert G, Koudinov A, et al.: Brain cholesterol, statins and Alzheimer's Disease. *Pharmacopsychiatry*. 2003; 36 Suppl 2: S113-S119.
32. Lutjohann D, Stroick M, Bertsch T, et al.: High doses of simvastatin, pravastatin, and cholesterol reduce brain cholesterol synthesis in guinea pigs. *Steroids*. 2004; 69: 431-438.
33. Lund E, Guileyardo J, Russell D: cDNA cloning of cholesterol 24-hydroxylase, a mediator of cholesterol homeostasis in the brain. *Proc Natl Acad Sci USA*. 1999; 96: 7238-7243.
34. Bjorkhem I, Meaney S, Diczfalusy U: Oxysterols in human circulation: Which role do they have? *Curr Opin Lipidol*. 2002; 13: 247-253.
35. Lutjohann D, Papassotiropoulos A, Bjorkhem I, et al.: Plasma 24S-hydroxycholesterol (cerebrosterol) is increased in Alzheimer and vascular demented patients. *J Lipid Res*. 2000; 41: 195-198.
36. Papassotiropoulos A, Lutjohann D, Bagli M, et al.: Plasma 24S-hydroxycholesterol: A peripheral indicator of neuronal degeneration and potential state marker for Alzheimer's disease. *Neuroreport*. 2000; 11:1959-1962.
37. Lutjohann D, von Bergmann K: 24S-hydroxycholesterol: A marker of brain cholesterol metabolism. *Pharmacopsychiatry*. 2003; 36 Suppl 2: S102-S106.
38. Wolozin B, Brown J, Theisler C, et al.: The cellular biochemistry of cholesterol and statins: Insights into the pathophysiology and therapy of Alzheimer's disease. *CNS Drug Review*. 2004; 10: 127-146.
39. Papassotiropoulos A, Streffer J, Tsolaki M, et al.: Increased brain beta-amyloid load, phosphorylated tau, and risk of Alzheimer disease associated with an intronic CYP46 polymorphism. *Arch Neurol*. 2003; 60: 29-35.
40. Desai P, DeKosky S, Kamboh M: Genetic variation in the cholesterol 24-hydroxylase (CYP46) gene and the risk of Alzheimer's disease. *Neurosci Lett*. 2002; 328: 9-12.
41. Kolsch H, Lutjohann D, Ludwig M, et al.: Polymorphism in the cholesterol 24S-hydroxylase gene is associated with Alzheimer's disease. *Mol Psychiatry*. 2002; 7: 899-902.
42. Mahley R, Innerarity T, Rall S, et al.: Plasma lipoproteins: Apolipoprotein structure and function. *J Lipid Res*. 1984; 25: 1277-1294.
43. Weisgraber K, Rall S, Mahley R: Human E apoprotein heterogeneity. Cysteine-arginine interchanges in the amino acid sequence of the apo-E isoforms. *J Biol Chem*. 1981; 256: 9077-9083.
44. Breslow JL: Genetic basis of lipoprotein disorders. *J Clin Invest*. 1989; 84: 373-380.
45. Herz J, Bock H: Lipoprotein receptors in the nervous system. *Ann Rev Biochem*. 2002; 71: 405-434.
46. Corder E, Saunders A, Strittmatter W, et al.: Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science*. 1993; 261: 921-923.
47. Roses A: Apolipoprotein E alleles as risk factors in Alzheimer's disease. *Ann Rev Med*. 1996; 47: 487-500.
48. Farrer L, Cupples L, Haines J, et al.: Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and

Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA*. 1997; 278: 1349-1356.
49. Schmechel DE, Saunders AM, Strittmatter WJ, et al.: Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein-E genotype in late-onset Alzheimer disease. *Proc Natl Acad Sci USA*. 1993; 90: 9649-9653.
50. Breitner J, Wyse B, Anthony J, et al.: APOE-epsilon4 count predicts age when prevalence of AD increases, then declines: The Cache County Study. *Neurology*. 1999; 53: 321-331.
51. Seshadri S, Drachman D, Lippa C: Apolipoprotein E epsilon 4 allele and the lifetime risk of Alzheimer's disease. What physicians know, and what they should know. *Arch Neurol*. 1995; 52: 1074-1079.
52. Slooter A, Cruts M, Kalmijn S, et al.: Risk estimates of dementia by apolipoprotein E genotypes from a population-based incidence study: The Rotterdam Study. *Arch Neurol*. 1998; 55: 964-968.
53. DeMattos R: Apolipoprotein E dose-dependent modulation of beta-amyloid deposition in a transgenic mouse model of Alzheimer's disease. *J Molec Neurosci*. 2004; 23: 255-262.
54. Ma J, Yee A, Brewer B, et al.: Amyloid-associated proteins alpha 1-antichymotrypsin and apolipoprotein E promote assembly of Alzheimer beta-protein into filaments. *Nature.* 1994; 372: 92-94.
55. Castano E, Prelli F, Wisniewski T, et al.: Fibrillogenesis in Alzheimer's disease of amyloid beta peptides and apolipoprotein. *Eur Biochem J*. 1995; 306: 599-604.
56. Bales K, Verina T, Cummins D, 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*. 1999; 96: 15233-15238.
57. DeMattos R, Cirrito J, Parsadanian M, et al.: ApoE and clusterin cooperatively suppress Abeta levels and deposition. Evidence that ApoE regulates extracellular Abeta metabolism in vivo. *Neuron*. 2004; 41: 193-202.
58. Bazin H, Marques M, Owens A, et al.: Inhibition of apolipoprotein E-related neurotoxicity by glycosaminoglycans and their oligosaccharides. *Biochemistry*. 2002; 41: 8203-8211.
59. Judson R, Brain C, Dain B, et al.: New and confirmatory evidence of an association between APOE genotype and baseline C-reactive protein in dyslipidemic individuals. *Atherosclerosis*. 2004; 177: 345-351.
60. Marz W, Scharnagl H, Hoffmann M, et al.: The apolipoprotein E polymorphism is associated with circulating C-reactive protein (the Ludwigshafen risk and cardiovascular health study). *Eur Heart J*. 2004; 25: 2109-2119.
61. Yasojima K, Schwab C, McGeer EG, et al.: Human neurons generate C-reactive protein and amyloid P: upregulation in Alzheimer's disease. *Brain Res*. 2000; 887: 80-89.
62. Mahley R, Rall S: Apolipoprotein E: far more than a lipid transport protein. *Ann Rev Genom Hum Genet*. 2000; 1: 507-537.
63. Davignon J, Gregg R, Sing C: Apolipoprotein E polymorphism and arteriosclerosis. *Arteriosclerosis*. 1988; 8: 1-21.