53 Trussell, L. O. and Fischbach, G. D. (1989) *Neuron* 3, 209–218
54 Borst, J. G. G., Lodder, J. C. and Kits, K. S. (1994) *J. Neurophysiol.* 71, 639–655
55 Thompson, S. M. and Gähwiler, B. H. (1992) *J. Neurophysiol.* 67, 1698–1701
56 Rogers, C. J., Twyman, R. E. and Macdonald, R. L. (1994) *J. Physiol.* 475, 69–82
57 Lambert, N. A. and Wilson, W. A. (1993) *Neuron* 11, 1057–1067
58 Samson, H. H. and Harris, R. A. (1992) *Trends Pharmacol. Sci.* 13, 206–211
59 Colquhoun, D. (1992) *Trends Pharmacol. Sci.* 13, 429–430
60 Segal, M. and Barker, J. L. (1984) *J. Neurophysiol.* 52, 469–487
61 Llano, I., Leresche, N. and Marty, A. (1991) *Neuron* 6, 565–574
62 Kano, M., Rexhausen, U., Dreessen, J. and Konnerth, A. (1992) *Nature* 356, 601–604
63 Cohen, G. A., Doze, V. A. and Madison, D. V. (1992) *Neuron* 9, 325–335
64 Mody, I., Tanelian, D. L. and MacIver, M. B. (1991) *Brain Res.* 538, 319–323
65 Buhl, E. H., Halasy, K. and Somogyi, P. (1994) *Nature* 368, 823–828
66 Manor, Y., Koch, C. and Segev, I. (1991) *Biophys. J.* 60, 1424–1437
67 Otis, T. S. and Mody, I. (1992) *J. Neurophysiol.* 67, 227–235
68 Hartzell, H. C., Kuffler, S. W. and Yoshikami, D. (1975) *J. Physiol.* 251, 427–463
69 Otis, T. S., De Koninck, Y. and Mody, I. (1994) *Proc. Natl Acad. Sci. USA* 91, 7698–7702
70 Valdes, F., Dasheiff, R. M., Birmingham, F., Crutcher, K. A. and McNamara, J. O. (1982) *Proc. Natl Acad. Sci. USA* 79, 193–197
71 McNamara, J. O. (1992) *Trends Neurosci.* 15, 357–359
72 Korn, H., Bausela, F., Charpier, S. and Faber, D. S. (1993) *J. Neurophysiol.* 70, 1249–1254
73 Kirsch, J., Wolters, I., Triller, A. and Betz, H. (1993) *Nature* 366, 745–748
74 Green, W. N., Ross, A. F. and Claudio, T. (1991) *Neuron* 7, 659–666
75 Otis, T. S., De Koninck, Y. and Mody, I. (1992) *Pharmacol. Commun.* 2, 75–83
76 Solis, J. M. and Nicoll, R. A. (1992) *J. Neurosci.* 12, 3466–3472
77 Segal, M. (1990) *Brain Res.* 511, 163–164
78 Sugita, S., Johnson, S. W. and North, R. A. (1992) *Neurosci. Lett.* 134, 207–211
79 Chu, D. C. M., Albin, R. L., Young, A. B. and Penney, J. B. (1990) *Neuroscience* 34, 341–357
80 Hökfelt, T. (1991) *Neuron* 7, 867–879
81 Baude, A. *et al.* (1993) *Neuron* 11, 771–787
82 Soltesz, I. *et al.* (1990) *Eur. J. Neurosci.* 2, 414–429
83 Soltesz, I. and Mody, I. (1994) *Soc. Neurosci. Abstr.* 20, 651
84 Vautrin, J., Schaffner, A. E. and Barker, J. L. (1992) *Neurosci. Lett.* 138, 67–71
85 Lisman, J. E. and Harris, K. M. (1993) *Trends Neurosci.* 16, 141–147
86 Spruston, N., Jaffe, D. B. and Johnston, D. (1994) *Trends Neurosci.* 17, 161–166
87 Pearce, R. A. (1993) *Neuron* 10, 189–200
88 Claiborne, B. J., Amaral, D. G. and Cowan, W. M. (1990) *J. Comp. Neurol.* 302, 206–219
89 Halasy, K. and Somogyi, P. (1993) *Eur. J. Neurosci.* 5, 411–429
90 Han, Z-S., Buhl, E. H., Lörinczi, Z. and Somogyi, P. (1993) *Eur. J. Neurosci.* 5, 395–410
91 Somogyi, P. and Soltesz, I. (1986) *Neuroscience* 19, 1051–1061
92 Gulyás, A. I. *et al.* (1993) *Nature* 366, 683–687
93 Libet, B., Gleason, C. A., Wright, E. W. J. and Feinstein, B. (1977) *Epilepsia* 18, 451–462
94 Madison, D. V. and Nicoll, R. A. (1988) *Brain Res.* 442, 131–138
95 Doze, V. A., Cohen, G. A. and Madison, D. V. (1991) *Neuron* 6, 889–900
96 Drewe, J. A., Childs, G. V. and Kunze, D. L. (1988) *Science* 241, 1810–1813
97 Akaike, N., Harata, N., Ueno, S. and Tateishi, N. (1992) *Brain Res.* 570, 102–108
98 Hosford, D. A. *et al.* (1992) *Science* 257, 398–401
99 Tossman, U., Jonsson, G. and Ungerstedt, U. (1986) *Acta Physiol. Scand.* 127, 533–545

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# Apolipoprotein E in animal models of CNS injury and in Alzheimer's disease

Judes Poirier

*Recent evidence indicates that apolipoprotein E (ApoE) plays a central role in the hippocampal response to injury. The co-ordinated expression of ApoE and its receptor, the ApoE/ApoB [low density lipoprotein (LDL)] receptor, appears to regulate the transport of cholesterol and phospholipids during the early and intermediate phases of the reinnervation process. During dendritic remodeling and synaptogenesis, neurons progressively repress the synthesis of cholesterol in favor of cholesterol internalization through the ApoE/LDL receptor pathway. The discovery that the ϵ4 allele is strongly linked to both sporadic and familial late-onset Alzheimer's disease (AD) raises the possibility that a dysfunction of the lipid-transport system associated with compensatory sprouting and synaptic remodeling could be central to the AD process. The role of ApoE in the CNS is particularly important in relation to the function of the cholinergic system, which relies to a certain extent on the integrity of phospholipid homeostasis in neurons. Recent evidence suggests that the ϵ4 allele has a direct impact on cholinergic function in AD.*

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Apolipoproteins are lipid carrier molecules that play a key role in regulating the metabolism of lipid following peripheral[1,2] and CNS injury[3–5]. Apolipoprotein E (ApoE) in particular is unique among apolipoproteins in that it has a special relevance to nervous tissue. It has been shown to co-ordinate the mobilization and redistribution of cholesterol in repair, growth and maintenance of myelin and neuronal membranes during development or after injury in the PNS[2,6,7]. In the CNS, ApoE plays a pivotal role in the mobilization and redistribution of cholesterol and phospholipid during membrane remodeling associated with synaptic plasticity[3–5] in the rat brain. In humans, the ϵ4 isoform has been shown to represent an important risk factor for late-onset sporadic[8,9] and familial Alzheimer's disease (AD)[10]. The absence of other key plasma apolipoproteins such as ApoA1 and ApoB (Ref. 11) in the brain further emphasizes the critical role of ApoE in the CNS.

## Apolipoprotein E

The mature form of ApoE present in human plasma and cerebrospinal fluid is a single glycosylated 37 kDa polypeptide containing 299 amino acids[12]. Apolipoprotein E is a constituent of several plasma lipoproteins [very low density lipoproteins (VLDL) and

| | ε4 | ε3 | ε2 |
|---|---|---|---|
| **Allele frequency on chromosome 19 in eastern Canada** | 0.152 | 0.770 | 0.078 |

| Phenotypes | | Relative charge | % | Isoelectric profile (- to +) |
|---|---|---|---|---|
| Homozygotes | ε4/4 | +2 | 3.9 | |
| | ε3/3 | +1 | 61.8 | |
| | ε2/2 | 0 | 2.0 | |
| Heterozygotes | ε4/3 | | 20.6 | |
| | ε4/2 | | 9.8 | |
| | ε3/2 | | 2.0 | |

| Protein coded by each allele (Apo E is 299 amino acids long) | ε4 | ε3 | ε2 |
|---|---|---|---|
| SITE 112 | ARG | CYS | CYS |
| SITE 158 | ARG | ARG | CYS |

**Fig. 1.** *Apolipoprotein E polymorphisms, population phenotype distribution and biochemical properties.*

high density lipoproteins (HDL)], and mediates the cellular uptake of lipid complexes through interaction with the ApoB/ApoE (LDL) receptor and distinct hepatic ApoE receptors[7]. Human ApoE is encoded by a four exon gene (3.6 kb in length) on the long arm of chromosome 19. Three major isoforms of ApoE (ϵ4, ϵ3 and ϵ2) differing by a single unit of net charge (Fig. 1), can be detected easily by isoelectrofocusing[13]. These isoforms are expressed from multiple alleles at a single ApoE genetic locus[14], giving rise to the three common homozygous phenotypes (ϵ4/4, ϵ3/3 and ϵ2/2) and three common heterozygous phenotypes (ϵ4/3, ϵ4/2 and ϵ3/2). Functionally, ϵ2 has a lower affinity for the LDL receptor than does ϵ3 and ϵ4 (Ref. 15). Lipoproteins associated with ϵ4 are cleared more efficiently than are those that contain ϵ3 and ϵ2. As a result, serum ApoE levels are lower in ϵ4 than in ϵ3 homozygotes. It has been estimated that 60% of the variation in serum cholesterol is genetically determined and that ApoE polymorphisms account for 15% of this genetic variation[16].

## Apolipoprotein E: a role in PNS regeneration and CNS reinnervation

When a peripheral nerve is sectioned or crushed, the distal segment undergoes typical functional and histological changes: myelin fibers begin to degenerate, and myelin ovoids are formed and converted into sudanophilic complexes (cholesterol and phospholipid enriched droplets). Cholesterol esters accumulate locally until regeneration and myelination are initiated. In response to the large amount of lipid released during this phase, macrophages synthesize and release ApoE within the peripheral lesion in order to scavenge cholesterol from both cellular and myelin debris[1,2]. As the intracellular content of cholesterol rises, cholesterol synthesis is depressed markedly through what appears to be a receptor-mediated downregulation of cholesterol synthesis initiated by internalization of lipoprotein[17]. Much of the cholesterol generated during this phase is stored within macrophages and apparently is re-used during subsequent axonal regeneration[18] and remyelination[2].

In the CNS, the ability of neurons to regenerate is very limited. However, specific brain areas have the ability to induce proliferation of presynaptic extensions from axons or terminals derived from undamaged neurons to compensate for the loss of specific input. A classical example of reactive synaptogenesis is illustrated by the compensatory response of the hippocampal formation to entorhinal-cortex lesions (ECL). Lesions to the entorhinal cortex have been shown to cause the loss of nearly 60% of the synaptic input to the granule-cell layer of the hippocampus. However, the loss of synapses is transient. Beginning a few days after denervation, new synapses are formed, virtually replacing the lost input within a few months[19].

This sequence of compensatory changes associated with ECL has been shown to coincide with the increased expression of ApoE in the deafferented zone of the molecular layer[3–5]. This is followed by an increase in LDL-receptor binding activity in granule-cell neurons undergoing dendritic proliferation and compensatory synaptogenesis[5]. In the deafferented hippocampus, cells that synthesize ApoE mRNA have been identified as astrocytes[4] rather than macrophages.

Figure 2 illustrates the postulated cascade of biochemical events that regulates the local recycling of cholesterol from degenerating terminals to neurons actively engaged in synapse remodeling and dendritic proliferation. Ultrastructural studies from Lee and colleagues[20] showed that throughout days 2–11 post-lesion (degeneration: days 0–5; reinnervation: days 5–60), astrocytes engulf progressively both presynaptic terminals and preterminal axons. Once metabolized, these terminal-derived ovoids generate a large glial store of lipids that are readily available for the synthesis of membrane components necessary for new synapses and dendrites. The accumulation of high levels of cellular cholesterol in astrocytes induces synthesis of ApoE, and its secretion with cholesterol. Cholesterol and ApoE are then combined into an as yet uncharacterized lipoprotein complex that resembles HDL (Ref. 21). The resulting ApoE–cholesterol complex might then be secreted into the circulation through overexpressed LDL receptors located in ependymal cells surrounding the third ventricle (Fig. 3) or directed to specific target sites within the CNS, or both. One of these targets has been identified as the granule-cell neuron of the dentate gyrus[5]. Granule-cell neurons in the hippocampus (Fig. 3) have been shown to exhibit a marked increase in number during LDL-receptor activity in the early and middle phases of the reinnervation process[5] (Fig. 2). Following binding of the ApoE complex with the LDL receptor, the ApoE–cholesterol–LDL receptor complex is internalized and degraded and the cholesterol is released within neurons (Fig. 2). The cholesterol can then be transported to the dendritic field (of granule-cell neurons) or to the terminals (of sprouting neurons in the molecular layer) for membrane and synapse formation. At six days post-lesion, during the early phase of the reinnervation process, accumulation of terminal-derived cholesterol in astrocytes and neurons of the injured hippocampal formation results in a

local reduction of the 3,3-hydroxymethylglutaryl-coenzyme A reductase (HMG-CoA reductase) activity[5] (Fig. 2), the rate-limiting step in the intracellular synthesis of cholesterol[22]. The apparent contradiction of depressed cholesterol synthesis and increased ApoE expression in the presence of active synaptogenesis can thus be reconciled by postulating a specific salvage and recycling of cholesterol from degenerating terminals through the pathway of ApoE transport and uptake of LDL receptors.

In support of this concept, Masliah[23] recently demonstrated that homozygous ApoE-deficient (knockout) mice display significant loss of synapses and marked disruption of the dendritic cytoskeleton with age. They also demonstrated poor hippocampal compensatory synaptogenesis following removal of entorhinal-cortex projections[23].

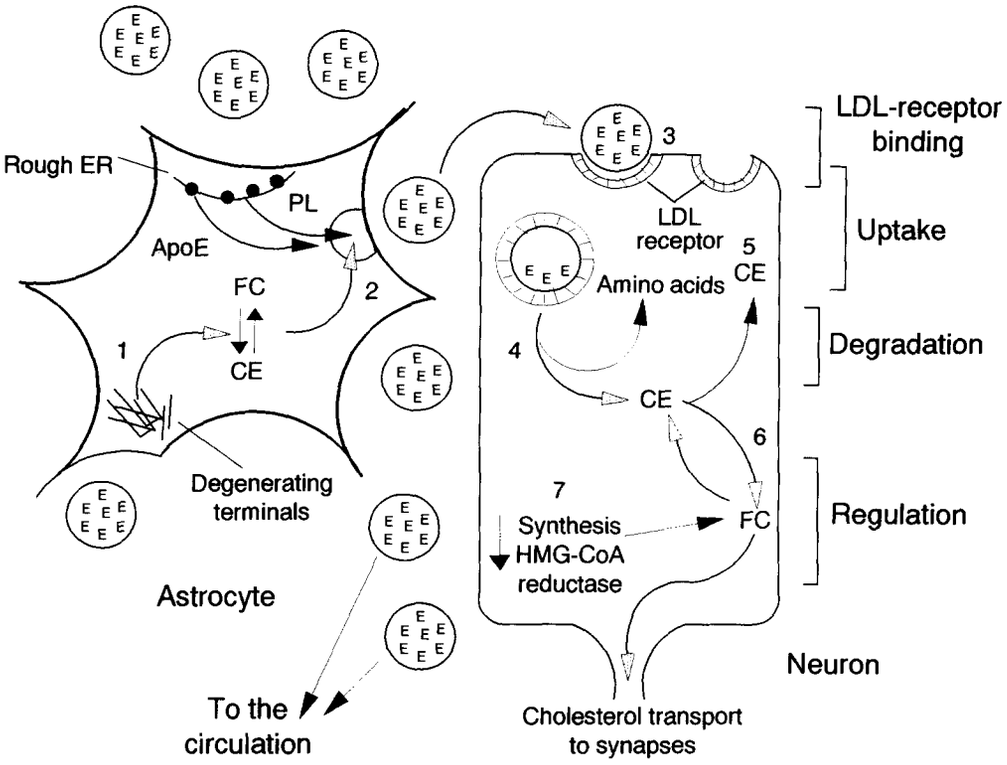


**Fig. 2.** *Schematic representation of hypothesized cholesterol–phospholipid recycling mechanisms in the injured CNS. Degenerating terminals are initially internalized and degraded. The non-esterified cholesterol (1) is used as free cholesterol (FC) for the assembly of an apolipoprotein E (ApoE)–cholesterol–lipoprotein complex (2) or converted into cholesterol esters (CE) for storage purposes. The newly formed ApoE–cholesterol–lipoprotein complexes are then directed toward the circulation, presumably through the ependymal cells surrounding the ventricles or to specific brain cells requiring lipids, or both. Apolipoprotein E complexes are apparently internalized by the neuronal low density lipoprotein (LDL) receptor pathway (3) and the cholesterol released (4) for dendritic proliferation or synaptogenesis, or both. As a consequence of the internalization process, cholesterol synthesis in neurons [via the 3,3-hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase pathway] becomes progressively repressed (7). Abbreviations: E, ApoE; and PL, phospholipids.*

## Synaptic plasticity and apolipoprotein E: a role in Alzheimer's disease?

Alzheimer's-disease (AD) pathology is characterized by an early and extensive loss of entorhinal-cortex neurons[24], and a marked loss of neurons in the CA1 area. In a rather limited number of patients with AD, the presence of regenerative changes in the dentate gyrus, as a consequence of the hippocampal deafferentation, has been documented[25,26]. This response is quite similar to the hippocampal response of rats following ECL. The proportion of patients with AD showing compensatory changes is still a controversial issue. By contrast, a marked reduction of the [$^3$H]glutamate-binding sites[27] and [$^3$H]kainate-binding sites[28] within the dentate gyrus of AD subjects has been reported while Ransmayr and colleagues[29] demonstrated a noticeable reduction of hippocampal choline acetyl transferease (ChAT) immunoreactivity in AD patients compared with control subjects. Electron microscopy studies by Flood and Coleman[30] have reported a loss of proliferating dendrites in the hippocampus in patients with AD while de Ruiter and Uyling[31] showed a loss of dendritic spines and decrease in spine length in the dentate of patients with AD. Taken together, these results suggest that the usual compensatory growth of fibers associated with hippocampal deafferentation appears somewhat compromised in most AD subjects.

Since ApoE has such a prominent role to play during reactive synaptogenesis in the injured rat brain, it is conceivable that the poor reinnervation reported in AD subjects could be the result of a selective impairment of the ApoE–LDL-receptor axis. Previous studies have shown that ApoE mRNA levels are increased[32] or unchanged[33] in post-mortem brains of AD patients whose ApoE genotype was unknown. A more recent study revealed a statistically significant correlation between the reduction in the hippocampal ApoE-protein content and the $\epsilon 4$ allele copy number (that is, as ApoE-protein content decreases, $\epsilon 4$ allele copy number increases: C. E. Finch, unpublished observations). Very recently, Nathan and colleagues[34] demonstrated that chicken neuronal cells in culture exposed to $\epsilon 3$ show enhanced neurite outgrowth, whereas cells exposed to $\epsilon 4$ exhibit decreased outgrowth compared with controls. The importance of ApoE in AD is further supported by its presence within plaques, neurofibrillary tangles and dystrophic neurites that characterize the neuropil pathology of AD[35,36], the ability of ApoE to bind the soluble and insoluble forms of β-amyloid with high affinity[37,38] and, the reported linkage between *ApoE* and *ApoCII* gene loci on chromosome 19 in some cases of late-onset familial AD (Ref. 39).

Assuming that ApoE is responsible, at least in part, for the poor compensatory response reported in AD subjects, then the integrity of the *ApoE* gene or its expression in AD, or both, has to be questioned.

## Apolipoprotein $\epsilon 4$ and Alzheimer's disease

Recently, several independent research groups have examined the frequency distribution of principal ApoE isoforms in AD and control subjects in the USA,

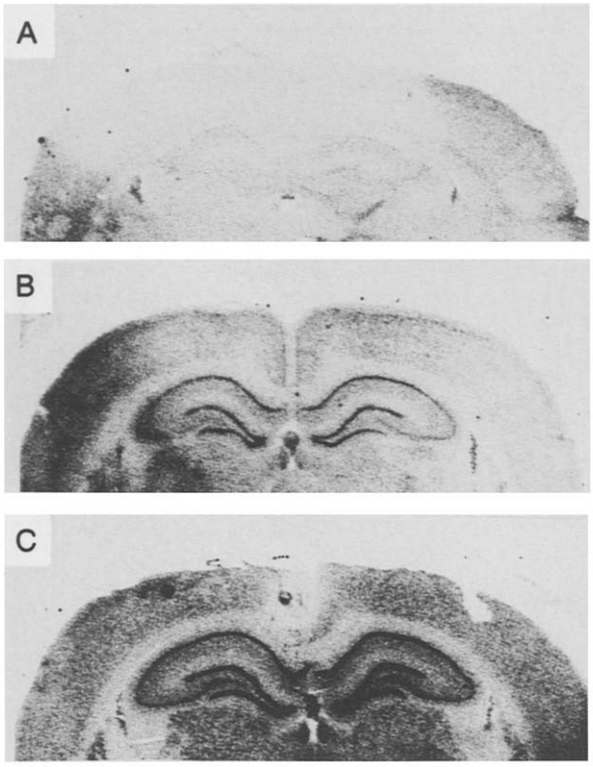


**Fig. 3.** *Autoradiographic analysis of [$^{125}$I]-LDL (low density lipoprotein)-receptor binding in the hippocampus of the rat following right unilateral entorhinal-cortex lesion (ECL).* **(A)** *Nonspecific binding.* **(B)** *LDL-receptor binding in control (sham-lesioned) rats and* **(C)** *LDL-receptor binding in ECL rats at 14 days post-lesion. Note the increased LDL-receptor binding at 14 days post-lesion in the ependymal cell layer surrounding the ventricular space as well as in granule cell neurons of the dentate gyrus and CA1 subfields on the lesioned right side.* Reproduced, with permission, from Ref. 5.

Canada, France, Japan and Germany. The frequency of the ϵ4 allele was shown to be markedly increased in sporadic[8,9,40,41] as well as in late-onset familial AD[10,42,43]. A gene-dosage effect was observed in both familial[10] and sporadic[8] cases (that is, as age of onset increases, ϵ4 allele copy number decreases).

Figure 4 represents the ϵ4-allele frequency and prevalence as a function of age distribution in living probable AD patients[8] and autopsy-confirmed AD subjects[44] from eastern Canada. As many as 80% of all clinically identified AD subjects between the age of 65 and 75 carry at least one copy of the ϵ4 allele. Interestingly, a sharp decline in the prevalence of the ϵ4 allele is observed in very old subjects (>85 years); suggesting the presence of a late-late onset form of AD. Comparable results have recently been obtained by Rebeck and colleagues[45] with a large clinical cohort of AD patients from the Boston area.

Control cases represent an interesting challenge. Living control subjects with mini-mental state scores greater than 28 (n = 74, mean age: 75.5 years) have been shown to exhibit an ϵ4-allele frequency of 12.2% (Ref. 8). Post-mortem control subjects with plaque and tangle counts below the AD threshold (n = 78, mean age: 70.2 years) exhibited an ϵ4-allele frequency of 5% (Ref. 44). Finally, living French centenarians (n = 325, mean age: 100.7 years), showing no signs of dementing illnesses, exhibited an ϵ4-allele frequency of less than 5% (Ref. 46). There is a marked reduction of the frequency of the ϵ4 allele when comparing cognitively normal octogenarians[8] (15.4%) with cognitively normal nonagerians[45] (5%) living in North America. Within the AD group, a marked enrichment of the ϵ4-allele frequency was observed in women compared with men[8]. At the phenotypic level, ϵ4/3 is more prevalent in women than men in AD, whereas the ϵ3/3 phenotype is under-represented in women compared with men[8]. The profile is consistent with the reported increased prevalence of AD in women compared with men in North America[47].

It is now clear that the inheritance of late-onset AD is associated with inheritance of the ϵ4 allele in individuals who live long enough to exhibit the disease. Thus, population frequencies of the ϵ4 allele might contribute to the prevalence of AD in different geographic locations and in genetically distinct populations. In this regard, Mayeux and his colleagues[48] have shown that the association between ϵ4 and AD seems to vary across three different ethnic groups (black, hispanic and white) living in New York City, USA.

Analysis of ϵ-allele frequencies in 45 populations shows that there are differences in ϵ-allele frequencies between Caucasian, Japanese and Chinese[49] populations. Although the clinical criteria used to define dementia might vary slightly between centers,

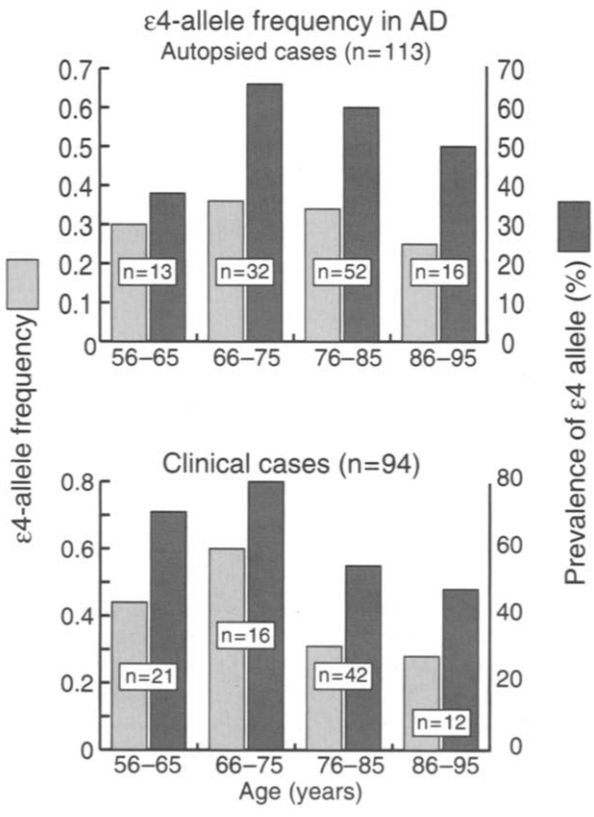


**Fig. 4.** *Apolipoprotein ϵ4-allele frequency and prevalence in autopsy-confirmed* **(top)** *and in clinical* **(bottom)** *cases of sporadic Alzheimer's disease (AD) from eastern Canada. Note the reduction in prevalence of the ϵ4 allele in both living and autopsied AD cases with age of onset greater than 85 years.* Adapted from Refs 8 and 44.

there is also a clear variation in the prevalence and incidence of AD around the world[50]. Hence, careful examination of the published ApoE phenotypes and genotypes for several different populations worldwide[49], and the age-adjusted prevalence of AD[50] reveals an interesting relationship (Fig. 5). As the frequency of the ε4 allele increases, the prevalence of AD also increases proportionally. In contrast, no correlation between the ε3- or ε2-allele frequencies and AD prevalence could be found in those same regions. This observation suggests that the well-established association between ε4 and AD is not population specific but represents a worldwide effect of genetic etiology.

## Amyloid plaques, neurofibrillary tangles and cholinergic dysfunction

Landmark pathological features of AD include generalized formation of plaques and neurofibrillary tangles and reduction in ChAT activity. Increased plaque density has recently been reported to occur in the cerebral cortex[51] and hippocampus (J. Poirier, R. Quirion, B. Gilfix and J. Nalbantoglu, unpublished observations) of subjects with late-onset AD who carry one or two copies of the ε4 allele.

A possible relationship between the ε4 genotype and cholinergic dysfunction in AD has also been examined recently in post-mortem brain tissues. As shown in Fig. 6, the reduction in ChAT activity in the hippocampus of patients with AD is proportional to the ε4 allele copy number (that is, as ε4 allele copy number increases, ChAT activity decreases)[53]. The loss of ChAT activity is apparently the result of a selective loss of the AChE-positive neurons in the diagonal band of Broca in Apoε4/4 compared with Apoε3/3 AD subjects (Fig. 6)[52].

A number of studies suggest that sporadic AD consists of distinct genetic entities showing differential impairment of cholinergic innervation, plaque density and neurofibrillary-tangle index. This intrinsic genetic susceptibility could result in subgroups of AD patients responding differently to cholinomimetics. The close relationship between ε4 genotype and reduction in residual ChAT activity suggests that ε4 genotype could serve to differentiate such subgroups. Clinical responsiveness to cholinergic agents monitored in genotyped AD patients might show that ε4 carriers are unlikely to be good responders, at least with the use of ACh precursors and AChE-based therapies.

## Toward a working model

Although ε4 is clearly associated with increased risk for developng AD, and decreased age of onset, the mechanism by which it operates is still unknown. Recently, Strittmatter and colleagues[54] proposed that isoform-specific interaction of ApoE with protein tau might alter neuronal tau metabolism and phosphorylation state in AD. It is suggested that this isoform-specific interaction could modulate the rate of formation of neurofibrillary tangles. In this model ε3, but not ε4, would make a bimolecular complex with tau that would prevent the abnormal phosphorylation of tau *in vivo*.

Alternatively, it was proposed several years ago[33] that the well-established role of ApoE in the transport of lipids is apparently compromised in the brain of AD

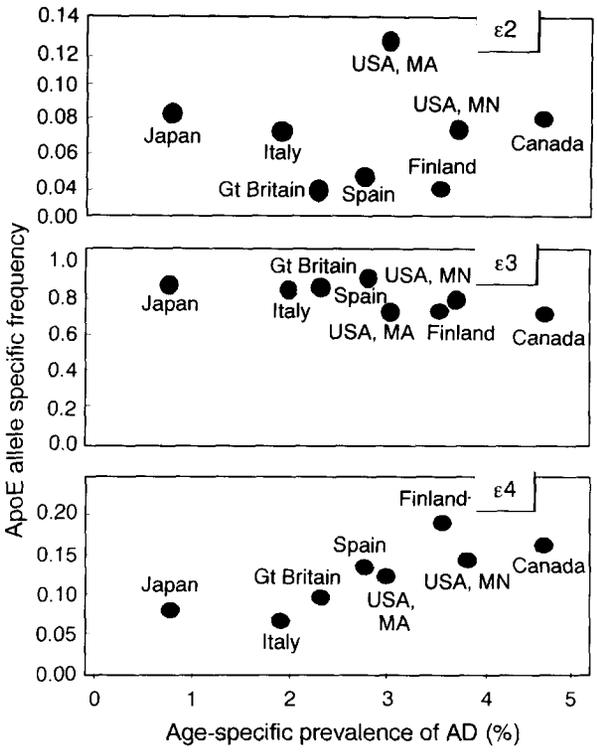


**Fig. 5.** *Schematic representation of the incidence of different apolipoprotein E (ApoE) polymorphisms as a function of age-adjusted prevalence for Alzheimer's disease (AD) in seven different countries and eight distinct regions.* Adapted from Refs 49 and 50.

subjects, resulting in an inefficient cholesterol and phospholipid transport and, ultimately, in a loss of synaptic integrity. Since age and age-related neuronal cell loss represent major risk factors in AD, it is postulated that cognitively intact elderly individuals exemplify subjects in which age-related neuronal cell loss is properly compensated for by a functional and efficient reinnervation process. Careful analyses of synaptic density in cognitively intact subjects reveal synaptic density similar to that found in young subjects, despite neuronal cell loss (for review, see Ref. 55). In contrast, compensatory synaptogenesis appears to be markedly compromised in most patients with AD. The co-ordinated expression of ApoE and its receptor during compensatory synaptogenesis in ECL rats, and the poor reinnervation response reported in ApoE-knockout mice, clearly establishes ApoE as a pivotal component in neuronal remodeling. Thus, it is quite conceivable that ε4, but not ε3, might interfere with the normal compensatory process occurring *in vivo*. Evidence supporting this idea has recently been demonstrated in cell culture experiments by Nathan *et al.*[34].

The cholinergic system relies heavily on an intact phospholipid metabolism[56], and this puts cholinergic neurons at high risk when compared with other cell types. In addition, selective vulnerability of the brain to injury could be explained, at least in part, by the absence of the usual backup apolipoproteins, such as ApoB and ApoAI, from the mammalian brain.

To summarize this model, ε4 AD carriers would suffer from poorly compensated age-related neuronal cell loss. The compensatory capacity of the brain

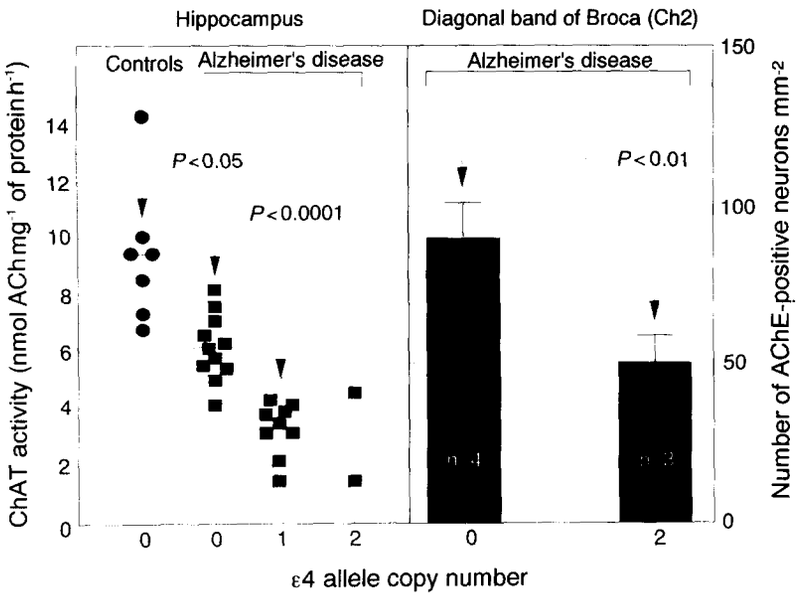


**Fig. 6.** *Apolipoprotein ε4 and cholinergic dysfunction in Alzheimer's disease (AD) subjects. Choline acetyltransferase (ChAT) activity was measured* **(left)** *in the hippocampus of post-mortem control and Alzheimer's disease subjects with different ApoE genotypes. Each point refers to one subject: control subjects are represented by solid circles; AD subjects are represented by solid squares. The AChE-positive neuron density in the diagonal band of Broca* **(right)** *was examined in homozygote ε3/3 and ε4/4 AD subjects. Significant differences between groups are indicated by the P values on the figure.* Adapted from Ref. 52.

would be correlated inversely with the ε4 allele copy number. This idea is consistent with ε4 homozygous AD subjects exhibiting an earlier age of onset than ε4 heterozygous or ε3 homozygous subjects.

## Concluding remarks

A detailed understanding of the pathophysiological role of ε4 in the CNS at the molecular level will most likely enable the design of novel therapeutic approaches to intervene early in the disease process. Furthermore, the expected development of transgenic mice overexpressing ε4 will certainly contribute to our understanding of the role of ApoE in normal and AD brains, as has the recent development of the ApoE-knockout mouse.

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## Selected references

1 Igniatus, M. J., Skene, J. H. P., Schilling, J. W., Weisgraber, K. H. and Shooter, E. M. (1986) *Proc. Natl Acad. Sci. USA* 83, 1125–1129
2 Boyle, J. K. *et al.* (1989) *J. Clin. Invest.* 83, 1015–1031
3 Poirier, J., Hess, M., May, P. C. and Finch, C. E. (1991) *Mol. Brain Res.* 9, 191–195
4 Poirier, J., Hess, M., May, P. C. and Finch, C. E. (1991) *Mol. Brain Res.* 11, 97–106
5 Poirier, J., Baccichet, A., Dea, D. and Gauthier, S. (1993) *Neuroscience* 55, 81–90
6 Muller, H. W., Igniatus, M. J., Hangen, D. H. and Shooter, E. M. (1986) *J. Cell Biol.* 102, 393–402
7 Mahley, R. W. (1988) *Science* 40, 622–630
8 Poirier, J., Davignon, J., Bouthillier, D., Bertrand, P. and Gauthier, S. (1993) *Lancet* 342, 697–699
9 Saunders, A. M. *et al.* (1993) *Neurology* 43, 1467–1472
10 Corder, E. H. *et al.* (1993) *Science* 261, 921–923
11 Roheim, P. S. (1979) *Proc. Natl Acad. Sci. USA* 76, 4646–4649
12 Rall, S. C., Weisgraber, K. H. and Mahley, R. W. (1982) *J. Biol. Chem.* 257, 4171–4178
13 Utermann, G., Pruin, N. and Steinmetz, A. (1979) *Clin. Genet.* 15, 37–62
14 Zannis, V. I. and Breslow, J. L. (1981) *Biochemistry* 20, 1033–1041
15 Weisgraber, K. H., Innerarity, T. L. and Mahley, R. W. (1982) *J. Biol. Chem.* 257, 2518–2521
16 Sing, C. F. and Davignon, J. (1985) *Am. J. Hum. Genet.* 37, 268–285
17 Goodrum, J. F. (1990) *J. Neurochem.* 54, 1709–1715
18 Rawlings, F. A., Villegas, G. M., Whyte, E. T. and Uzman, B. G. (1972) *J. Cell Biol.* 52, 615–625
19 Matthews, D. A., Cotman, C. W. and Lynch, G. (1976) *Brain Res.* 115, 1–41
20 Lee, K. S., Standford, E. J., Cotman, C. W. and Lynch, G. S. (1977) *Exp. Brain Res.* 29, 475–485
21 Chiba, H. (1991) *Neurosci. Lett.* 113, 207–210
22 Brown, M. S., Dana, S. E. and Goldstein, J. L. (1973) *Proc. Natl Acad. Sci. USA* 70, 2162–2166
23 Masliah, E. in *Proceedings of the Third International Conference on Alzheimer's Disease* (Iqbal, K., ed.), Wiley (in press)
24 Hyman, B. T., Van Hoesen, G. W., Damasio, A. R. and Barnes, C. L. (1984) *Science* 225, 1168–1170
25 Geddes, J. W. *et al.* (1985) *Science* 230, 1179–1181
26 Hyman, B. T., Kromer, L. J. and Van Hoesen, G. W. (1987) *Ann. Neurol.* 21, 259–267
27 Greenamyre, J. T., Penney, J. B., D'Amato, C. B. and Young, A. B. (1987) *J. Neurochem.* 84, 543–551
28 Represa, A., Duyckaerts, C., Tremblay, E., Hauw, J. J. and Ben-Ari, Y. (1988) *Brain Res.* 457, 355–359
29 Ransmayr, G., Hisch, E. and Agid, Y. (1989) *Neuroscience* 32, 701–714
30 Flood, D. G. and Coleman, P. D. (1987) *Can. J. Neurol. Sci.* 13, 475–479
31 de Ruiter, J. P. and Uyling, H. B. M. (1987) *Brain Res.* 402, 217–229
32 Deitrich, J. F., Minnigan, H., Carp, R. I. and Haase, A. T. (1991) *J. Virol.* 65, 4759–4768
33 Poirier, J., Hess, M., May, P. C., Pasinetti, G. and Finch, C. E. (1990) in *Basic and Therapeutic Strategies in Alzheimer's and Parkinson's Diseases* (Nagatsu, T., ed.), pp. 191–194, Plenum Press
34 Nathan, B. P., Bellosta, S., Sanan, D. A., Weisgraber, K. H. and Pitas, R. E. (1994) *Science* 264, 850–852
35 Namba, Y. (1991) *Brain Res.* 541, 163–166
36 Wisniewski, T. and Frangione, B. (1991) *Neurosci. Lett.* 135, 235–238
37 Wisniewski, T. and Frangione, B. (1992) *Biochem. Biophys. Res. Commun.* 192, 359–365
38 Strittmatter, W. J. *et al.* (1993) *Proc. Natl Acad. Sci. USA* 90, 1977–1981
39 Schellenberg, G. D. *et al.* (1992) *Ann. Neurol.* 31, 223–227
40 Lucotte, G., David, F., Babron, M. C. and Couderc, R. (1993) *Lancet* 342, 1309
41 Czech, C. *et al.* (1993) *Lancet* 342, 1309–1310
42 Strittmatter, W. J. *et al.* (1993) *Proc. Natl Acad. Sci. USA* 90, 1977–1981
43 Payami, H., Kaye, J. and Heston, L. L. (1993) *Lancet* 342, 738
44 Nalbantoglu, J. *et al. Ann. Neurol.* (in press)
45 Rebeck, G. W., Perl, T. T., West, H. L. and Hyman, B. T. (1994) *Neurology* 44, 1513–1516
46 Schachter, F. and Cohen, C. (1994) *Nature Genet.* 6, 29–31
47 Canadian Study of Health and Aging Working Group (1994) *Can. Med. Assoc. J.* 150, 899–906
48 Mayeux, R. *et al.* (1993) *Ann. Neurol.* 34, 752–754
49 Gerges, L. U., Klausen, I. C. and Faergeman, O. (1992) *Genet. Epidemiol.* 9, 155–179
50 Breteler, M. M. B. (1992) *Epidemiol. Rev.* 14, 59–93
51 Schmechel, D. E. *et al.* (1993) *Proc. Natl Acad. Sci. USA* 90, 9649–9654
52 Poirier, J. *et al.* in *Advances in Alzheimer Therapy* (Giacobini, E. and Becker, R., eds), Birkhäuser (in press)
53 Poirier, J., Nalbantoglu, J., Guillaume, D. and Bertrand, P. in *Neurofibrillary Tangles and Amyloid Protein in Alzheimer's Disease* (Goate, A. and Ashall, F., eds), Academic Press (in press)
54 Strittmatter, W. J., Weisgraber, K. H., Goedert, K. H. and Roses, A. D. (1994) *Exp. Neurol.* 125, 163–171
55 Poirier, J. and Finch, C. E. (1993) in *Principles of Geriatric Medicine and Gerontology* (3rd edn), (Hazzard, W. R., Andres, R., Bierman, E. L. and Blass, J. P., eds), pp. 1105–1112, McGraw Hill
56 Wurtman, R. J. (1992) *Trends Neurosci.* 15, 117–122