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The apolipoprotein E ε4 allele plays pathological roles in AD through high protein expression and interaction with butyrylcholinesterase

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Abstract

The apolipoprotein E (ApoE) ε4 allele has consistently been established as an Alzheimer's disease (AD) risk factor, but its pathological contribution to AD is obscure. Certain butyrylcholinesterase (BuChE) polymorphisms together with the ApoE ε4 allele synergistically increase the risk of AD. In addition, AD risk factors, i.e. advanced age, female gender and ApoE ε4 are associated with different levels of CSF BuChE in AD patients, and BuChE protein attenuates Aβ fibrillization *in vitro*. Here we investigated the roles of ApoE and BuChE gene products as modulators of pathological features of AD *in vivo*. We found that AD risk factors were associated with different levels of ApoE protein in the CSF of AD patients ($n = 115$). Women and ApoE ε4 carriers had the highest levels of ApoE protein (up by 50–120%, $p < 0.01$–0.0001), which were increased with age ($r = 0.30$, $p < 0.0006$). The CSF surrogate markers of pathological features of AD, i.e. high tau and P-tau, low $A\beta_{42}$ and high tau/$A\beta_{42}$ ratio, were associated with high levels of ApoE protein. Intriguingly, high ApoE protein levels were not only associated with low amounts of BuChE, but they also altered the aging and activity of this enzyme in concentration- and isoform-dependent manners, particularly in the presence of Aβ peptides. Both ApoE and BuChE levels were also differentially related to levels of the proinflammatory cytokine IL-1β. In conclusion, ApoE ε4 might impart its pathological role through high protein expression and interaction with BuChE, which in turn might modulate central cholinergic activity and Aβ load in the brain.

Keywords: Alzheimer's disease; Apolipoprotein E; Butyrylcholinesterase; Risk factors; Beta amyloid; Fibrillization; Proinflammatory cytokine (IL-1β)

1. Introduction

Alzheimer's disease (AD) is one of the most devastating neurodegenerative disorders, which yearly afflicts 4.6 million patients worldwide. The major risk factors of AD are advanced age, female gender and the apolipoprotein E (ApoE) ε4 allele. Other candidates also exist such as interleukin-1 (IL-1) gene polymorphism and low-density lipoprotein receptor (LDLR)-related protein (LRP) (Herz and Beffert, 2000).

Apolipoprotein E protein plays an important role in the metabolism, transport and redistribution of cholesterol carriers and other lipids which are used for membrane synthesis as well as for other cellular anabolic and catabolic activities throughout the body, including those of the CNS, a site of high lipid turnover (Poirier, 2005). ApoE protein serves as a ligand for the cell-surface LDLR family, such as LDLR, VLDLR and their related receptors, LRP1, LRP2 and ApoE receptor-2 (Beffert et al., 2004). In the brain, LDLR and LRP1 are the principal metabolic receptors for ApoE lipoprotein particles (Fryer et al., 2005; Liu et al., 2007). LRP1 is mainly expressed by neurons, whereas LDLR is expressed by glial cells (Fryer et al., 2005; Liu et al., 2007).

Several observations suggest functional differences between ApoE isoproteins. Lipidated ApoE ε4 is less efficient than ε3-isoprotein in cholesterol transport in the brain

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(Rapp et al., 2006) or in promoting cholesterol efflux from neurons and astrocytes (Michikawa et al., 2000). In primary neurons, ApoE ε4, but not the ε3 isoform increases neurotoxicity via LRP1 (Qiu et al., 2003) and neurons seem to be less protected against apoptosis by glial ApoE ε4 lipoproteins compared with ε3 lipoproteins (Hayashi et al., 2007).

Despite the fact that the ApoE ε4 allele is the only genetic factor recurrently documented to increase the risk of developing AD, its pathological role in AD and its interaction with the pathological markers of AD, such as fibrillization and oligomerization of beta amyloid (Aβ) peptides and hyperphosphorylation of tau protein is very obscure. *In vitro* studies suggest that synthetic Aβ peptides readily form complexes with human ApoE protein, in particular the ε4 isoform (Strittmatter et al., 1993a, b; Sweeney et al., 2004). ApoE ε4 appears to modulate amyloid precursor protein (APP) processing and Aβ production through both the LRP pathway and intramolecular domain interaction (Ye et al., 2005).

Besides ApoE protein, the cholinergic enzyme acetylcholinesterase (AChE) and the related enzyme butyrylcholinesterase (BuChE) are found within Aβ deposits (plaques) in the AD brain (Inestrosa et al., 1996). Intriguingly, in contrast to other neurotransmitter systems, the cholinergic system relies heavily on lipids for the synthesis of its neurotransmitter, acetylcholine (ACh) (Poirier, 2005) and the central cholinergic system is one of the first neuronal networks that is heavily affected by AD (Davies and Maloney, 1976). The activity of cholinergic neurons also shows an age-dependent decrease (Davies and Maloney, 1976). The enzymes AChE and BuChE catalyze the hydrolysis of ACh, a key process in the regulation of cholinergic neurotransmission. Owing to the marked deficit of cholinergic neurotransmission in AD and its correlation with memory and cognitive impairment in these patients, both enzymes are more or less the primary targets of current therapy with cholinesterase inhibitors (ChEIs). This is to reduce the symptoms of the disease by inhibiting these enzymes and hence prolonging the action of ACh at synapses of the remaining functional neurons.

Postmortem brain studies indicate that the level of BuChE substantially increases in the hippocampus with age (Perry et al., 1978), as well as in the AD brain compared with controls (Arendt et al., 1992). Interestingly, BuChE reactivity seems to differentiate between Aβ plaques associated with normal aging from those associated with dementia (Mesulam and Geula, 1994), leading to the suggestion that BuChE may facilitate transformation of an initially benign form of Aβ deposit to malignant forms associated with neuritic tissue degeneration and clinical dementia (Mesulam and Geula, 1994; Guillozet et al., 1997). Interestingly, this is in agreement with the observation that increased levels of BuChE in the gray matter of the temporal cortex are linked to the steep annual decline in cognitive performance of patients with dementia (McKeith et al., 2003). However, the substantial increases of BuChE levels in the AD brain are in strong contrast to the reductions observed in the CSF (Appleyard et al., 1987; Mesulam and Geula, 1994) and in the synaptic regions as assessed by positron emission tomography (PET) (Kuhl et al., 2006). We have shown that the three main AD risk factors are associated with different levels of BuChE activity in the CSF of AD patients (Darreh-Shori et al., 2006). CSF BuChE activity is higher in men than in women, in ApoE ε4-negative patients than in those carrying one or two ε4 alleles, and it decreases with age (Darreh-Shori et al., 2006). We have also shown that BuChE levels correlate positively with cortical glucose utilization, and patients with high to moderate CSF BuChE levels show better cognitive function scores than other AD patients (Darreh-Shori et al., 2006). In addition, we have shown a synergistic effect between the BuChE-K variant and ApoE ε4 on the incidence of AD in subjects with mild cognitive impairment (MCI) and the degree of hippocampal atrophy, which inversely correlated with the activity of BuChE in the patients (Lane et al., 2008). The results of *in vitro* studies have also suggested a attenuating effect of BuChE or its C-terminal peptide on fibrillization of synthetic Aβ peptides (Diamant et al., 2006; Podoly et al., 2008).

Inflammation clearly occurs in the AD brain, through activation of microglial cells in the CNS (Zhu et al., 1999). Variable numbers of activated microglial cells and astrocytes are often situated in the vicinity of the fibrillar core of Aβ plaques (Braak et al., 1998). In addition, inflammation can also markedly alter memory function, which can be observed in healthy subjects following endotoxin injection or in patients receiving cytokine immunotherapy for cancer (Meyers, 1999). Intriguingly, acetylcholine binds to α7-nicotinic acetylcholine receptors (nAChRs), expressed by macrophages, and this suppresses the production and release of proinflammatory cytokines (e.g. IL-1β, IL-6 and TNF-α) (Wang et al., 2003). Changes in ACh-hydrolyzing capacities, i.e. AChE and BuChE activities in the brain parenchyma, may therefore affect regulatory mechanisms involving inflammatory responses in the brain during the natural course of AD as well as following treatment of AD patients with cholinesterase inhibitors (ChEIs).

The lack of a good and validated animal model is one of the most important obstacles in the field of research into Alzheimer's disease. In this study, we focused our investigation on CSF and plasma samples from 115 AD patients. We determined the levels of ApoE protein and the proinflammatory cytokines IL-1β and IL-6. Next, we investigated their interrelationships with levels of BuChE, tau and Aβ peptides in the CSF, to provide a mechanistic understanding of *ApoE* and *BuChE* gene products as modulators of the pathological features of AD in the brains of living patients.

2. Material and methods

2.1. Patients and CSF samples

Samples of CSF from 115 patients with probable AD and from one with MCI were available and were included in