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Effect of apolipoprotein E (apoE) phenotype on the apoE content of CSF-HDL in children

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Abstract

Background: The majority of the lipoprotein in cerebrospinal fluid (CSF) is apolipoprotein E (apoE)-containing HDL. Since neuronal cells express lipoprotein receptors which recognize apoE, apoE in CSF-HDL is believed to be important for the development of central nervous system (CNS) in children. In adults, the apoE phenotype affects the plasma apoE concentration and the ε 4 allele is a risk factor for Alzheimer's disease. Due to the requirement for CNS development, we examined whether the apoE phenotype affects the composition and concentration of CSF-HDL in children.
Methods: We determined the apoE phenotype in 107 neurologically normal subjects, including 67 children (<20 years), by isoelectronic focusing. We also measured apoE, total cholesterol (TC), and phospholipid (PL) concentrations in the CSF.
Results: The respective frequencies of apoE4/3, E3/3 and E3/2 were 16.4%, 77.6%, and 6.0%. The allele frequencies of ε 4, ε 3, and ε 2 were 0.082, 0.888, and 0.030, respectively. There were no significant differences in the CSF-apoE, TC, or PL concentrations or the apoE/PL ratio among the apoE phenotypes. However, the CSF-apoE/PL ratio was significantly higher in children than in adults.
Conclusion: The apoE phenotype does not affect the composition or concentration of CSF-HDL in children. We speculate that an apoE4 carrier is prevented in childhood from the impaired development of central nervous system by CSF-HDL enriched with apoE.

Keywords: Apolipoprotein E (apoE); apoE phenotypes; LDL receptor family; Cerebrospinal fluid (CSF)-HDL; CNS development; Alzheimer's disease

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1. Introduction

Apolipoprotein E (apoE) is a 35-Da glycoprotein that binds to the LDL receptor family, including the LDL receptor, LDL receptor-related protein (LRP), LR11/SorLA, VLDL receptor/apoE receptor (apoER), and apoE receptor 2 (apoER2). In plasma, apoE is distributed over several lipoprotein classes. Among them, apoE is crucial for the uptake of remnant lipoproteins from the circulation by the liver. The cerebrospinal fluid (CSF)/serum ratio of albumin is 1/200 to 1/400 due to the blood-brain barrier, while that of apoE is as much as 1/10 to 1/20. Such enrichment of apoE in CSF is understandable, because apoE is synthesized in neuronal cells, glia cells, astrocytes, and macrophages in the central nervous system (CNS). In CSF, apoE is associated with HDL, which is larger than that of plasma HDL [1].

Experimental studies have shown that apoE plays an important role in neurite outgrowth and neuron repair [2–4]. Interestingly, several members of the LDL receptor family, including LRP, LR11, apoER, and apoER2, are strongly expressed in the brain [5,6]. They probably contribute to lipid transport and metabolism in the CNS via receptor-mediated endocytosis [7]. Recently, some have been shown to be involved in the Reelin signaling pathway and to play roles in neuronal cell migration in the brain [8,9]. Since apoB is not detectable in the CSF, apoE is likely the main ligand for the LDL receptor family that organizes cholesterol and phospholipid transport in the CNS [1,8,9].

ApoE has 3 major isoforms (E2, E3, and E4) that are associated with hypercholesterolemia or familial type III hyperlipidemia [10,11]. Therefore, the apoE phenotype clearly affects the plasma lipoprotein profile. Several epidemiological studies have shown that the ε 4 allele of apoE is a risk factor for both sporadic and late-onset familial Alzheimer's disease [12–14]. ApoE4 intensifies all the biochemical disturbances characteristic of AD, including β amyloid deposition, tangle formation, neuronal cell death, oxidative stress, impaired synaptic plasticity, and dysfunction of lipid homeostasis and cholinergic signaling [15,16].

Due to the suspected importance of CSF-apoE, several groups have investigated the clinical relevance of the CSF-apoE concentration and apoE phenotype in neurological diseases [17–19]. However, consistent results have not been obtained. Previously, we have reported that the apoE content of CSF-HDL was significantly higher in children than in adults [20]. Such enrichment of apoE in CSF-HDL may benefit the organization and development of the CNS in children. Since mental retardation has not been reported in apoE4 carriers, we postulated that the apoE content of CSF-HDL is not affected by the apoE phenotype in children. Therefore this study examined whether the apoE phenotype affects the apoE content of CSF in children. To address this question, we determined the apoE phenotype and concentration of CSF-HDL components in the clinically normal children and adults, and the effect of apoE phenotype on the apoE content of CSF-HDL.

2. Materials and methods

2.1. Subjects

CSF samples were collected from 107 subjects without neurological disorders (70 males and 37 females, aged 1–86 years), who had a lumbar puncture to exclude CNS infection (n=8), for lumbar anesthesia (n=43), or for intrathecal chemotherapy for leukemia during the stage of complete remission (n=56). Informed consent was obtained from all of the subjects, (and their parents in the case of children). We excluded infants (<12 months) since their blood-brain barrier is immature [21], and samples from this study if the spinal tap was traumatic. In all the CSF samples, the laboratory values, including cell counts and glucose, protein, and electrolyte concentrations, were within the reference intervals (data not shown).

2.2. Agarose gel electrophoresis

To determine the distribution of apolipoproteins in the CSF, fresh samples were separated in a 0.75% agarose gels. Then, they were electrically transferred onto a nitrocellulose (NC) sheet (Trans-Blot Transfer Medium, BioRad, Hercules, CA) [22]. The CSF-lipoproteins on the NC sheets were immunoblotted with polyclonal antibodies against the apolipoproteins of interest, and the corresponding secondary antibodies complexed with horse-radish peroxidase. The apolipo-