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High apolipoprotein E in cerebrospinal fluid of patients with Lewy body disorders is associated with dementia

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Abstract Apolipoprotein E ε4 allele (*APOE* ε4) increases the apolipoprotein E (apoE) protein levels in Alzheimer's disease (AD) cerebrospinal fluid (CSF). Thus, we hypothesized that apoE levels were also associated with the *APOE* genotype, and amyloid-β (Aβ)-associated clinical, functional, and imaging parameters in patients with Lewy body-associated disorders (LBD). Indeed, similar to AD, patients with LBD displayed high CSF apoE levels (greatest in patients with dementia with LBD), and this was linked to *APOE* ε4. High CSF apoE protein correlated positively with CSF soluble amyloid precursor protein, total tau, and cortical and striatal Pittsburgh compound B retention; and correlated negatively with CSF $A\beta_{42}$, cognitive tests scores, and glucose uptake ratio in the temporal and parietal cortices. *APOE* ε4-triggered accumulation of apoE in CSF is related to Aβ-associated clinical and functional imaging parameters in LBD. Accordingly, therapeutic strategies aimed at reducing apoE levels in the brain should be explored not only in AD but also in LBD, particularly when accompanied with dementia.

Keywords: Lewy body-associated disorders; Dementia with Lewy bodies; Alzheimer's disease; Cerebrospinal fluid; Apolipoprotein E; Amyloid-β; Positron emission tomography; Fluorodeoxyglucose–positron emission tomography; Pittsburgh compound B–positron emission tomography

1. Introduction

The term *Lewy body-associated disorders* (LBD) is used as an umbrella term for patients with Parkinson's disease (PD) and dementia with Lewy bodies (DLB). Among cases of PD, dementia is common, with a cumulative prevalence of about 80% [1], and patients with PD with dementia (PDD) share many features with DLB [2]. In the majority of the patients with dementia with LBD, the pathological correlates of dementia are proposed to be Lewy bodies outside the substantia nigra as well as Alzheimer's disease (AD)-type pathology [3,e1,e2].

A common feature between AD and DLB is amyloid β (Aβ) peptide deposition in the brain [3,e1,e2]. Aβ peptides are generated by one of the two proteolytic processes of amyloid precursor protein (APP). Enzymatic cleavage of APP by β-secretase generates Aβ peptides and soluble APPβ (sAPPβ) [4], which is referred to as the amyloidogenic pathway [4]. The nonamyloidogenic pathway is instead mediated by the α-secretases, which cleave APP within the Aβ domain, generating the longer sAPPα but no full-length Aβ peptides [4].

Another common feature between AD and LBD is that cholinergic neurotransmission is one of the first neuronal networks to become heavily affected [5]. A key process in the regulation of cholinergic neurotransmission is the hydrolysis of acetylcholine by acetylcholinesterase (AChE). In patients with AD, the relative cerebrospinal fluid (CSF) concentrations of AChE splice variants are altered, so that

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the ratio of the read-through AChE and the synaptic AChE (AChE R/S) splice variants is decreased with time in untreated patients with AD [6,7]. In contrast, an increase in the AChE R/S in response to treatment with cholinesterase inhibitors also shows a highly significant correlation with patients' cognition [6,7]. The AChE and butyrylcholinesterase proteins with altered enzymatic properties, such as major shifts in optimum pH and sensitivity to various inhibitors, are found within Aβ deposits (plaques), neurofibrillary tangles, and cerebral amyloid angiopathy in the AD brain [8,e3–e6].

Another protein detected in Aβ deposits is apolipoprotein E (apoE). apoE protein is a major lipoprotein in the brain. In humans, apoE is expressed as three isoforms—apoE2, 3, and 4—where *APOE* ε3 is the most common allele [9,e7]. *APOE* ε4 is the strongest genetic risk factor for early- and late-onset of AD with a two- to threefold increase in risk in individuals expressing one ε4 allele and a 10-fold increase in subjects with two ε4 alleles [10,e8]. The frequency of *APOE* ε4 is significantly higher in patients with PDD compared with patients with PD without dementia (PDND) and older persons without a neurodegenerative disease [11]. It is also increased in patients with DLB with co-occurring AD-type pathology, but not in those without [12].

In patients with AD, *APOE* ε4 is associated with increased amyloid β (Aβ) deposition [13], and in vitro studies suggest that synthetic Aβ peptides bind readily to the human apoE protein, in particular the ε4 isoform [e9,e10]. In addition, evidence based on animal studies supports the proposed pathological interactions between Aβ and the apoE protein [14,e9,e11–e14] leading to cognitive deficits [15,e15]. These observations indicate that *APOE* ε4 potentiates the neurotoxic effects of Aβ. However, the short- and long-term molecular mechanisms underlying the pathological cross-talk between apoE protein and Aβ peptides, and the extent to which they also mediate other pathological hallmarks of *APOE* ε4 in AD—such as impaired neuronal plasticity and repair [16,e16] and increased brain inflammation [17]—are currently unclear.

Studies in patients with AD links the *APOE* ε4 genotype to high levels of apoE protein in CSF [18]. High apoE protein levels have in turn been tied to high CSF phosphorylated tau levels and Aβ load, and low glucose use in vivo in the brain of patients with AD [19]. These findings have been confirmed recently in a mouse model of Aβ amyloidosis, in which genetic manipulation of the *APOE* gene dosage demonstrated that decreasing human apoE levels, regardless of isoform status, results in significantly decreased Aβ plaque deposition and microglial activation [20].

Because apoE protein is involved crucially in the pathogenic processes in AD, and AD-type pathologies are associated tightly with cognitive decline in LBD, we hypothesized that CSF apoE protein may also be related to Aβ-associated parameters in LBD.

2. Methods

2.1. Participants

This patient cohort has been described earlier [21]. Briefly, 28 patients with LBD (nine with DLB, nine with PDD, and 10 with PDND) fulfilling clinical criteria for PD [22] or DLB [23] were recruited by experienced neurologists (D.B, W.M.) from the Neurodegenerative Department, University of Tuebingen (Tuebingen, Germany) using a consequent and standardized clinical examination procedure, with which an accuracy of more than 90% for the correct diagnosis can be expected [24]. In addition, an extensive neuropsychological test battery was performed. Diagnosis of PDD was made according to the clinical diagnostic criteria of the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition [25], indicating cognitive impairment in at least two domains, including memory dysfunction and the clinically rated impact on activities of daily living function. The clinical rating was based on patients' or caregivers' reports of a marked activity of daily living dysfunction caused primarily by cognitive worsening. Differentiation of DLB from PDD was done with the 1-year rule—in other words, PDD was diagnosed if dementia occurred later than 1 year after the first occurrence of motor symptoms; DLB was diagnosed if dementia occurred no later than 1 year after the first occurrence of motor symptoms or even preceded motor symptoms [23].

All participants underwent magnetic resonance imaging shortly before the study to exclude atrophy and severe deep white matter lesions, which are typical signs for vascular pathology. All participants underwent the assessments reported here within the time frame of 1 month.

The local ethics committee approved the study, and informed consent was obtained from all participants.

2.2. CSF collection and analyses

CSF was taken from all subjects by lumbar puncture using polypropylene tubes. Routine CSF diagnostics (cell count, albumin, immunoglobulin G level, immunoglobulin G index, and cytology) were without significant differences among DLB, PDD, and PDND subjects [26]. CSF samples were centrifuged within 1 hour after collection and stored at −70°C until analysis.

CSF apoE protein was measured by sandwich enzyme-linked immunosorbent assays (ELISAs) as described previously [18]. The protein concentration of the AChE variants (AChE synaptic and AChE read-through) was determined as reported previously [27].

Total tau and phosphorylated tau 231 levels in undiluted CSF samples was determined using a duplex ELISA kit for total tau/phosphorylated tau 231 from Meso Scale Discovery, Rockville, MD, USA (96-well *MULTI-SPOT* AD assay: phosphorylated (Thr 231)/total tau with purified neuronal tau calibrators). sAPPα and -β levels in 50× diluted samples