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Functional variability in butyrylcholinesterase activity regulates intrathecal cytokine and astroglial biomarker profiles in patients with Alzheimer's disease

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ABSTRACT

Butyrylcholinesterase (BuChE) activity is associated with activated astrocytes in Alzheimer's disease brain. The BuChE-K variant exhibits 30%–60% reduced acetylcholine (ACh) hydrolyzing capacity. Considering the increasing evidence of an immune–regulatory role of ACh, we investigated if genetic heterogeneity in BuChE affects cerebrospinal fluid (CSF) biomarkers of inflammation and cholinoceptive glial function. Alzheimer's disease patients ($n = 179$) were *BCHE–K*-genotyped. Proteomic and enzymatic analyses were performed on CSF and/or plasma. BuChE genotype was linked with differential CSF levels of glial fibrillary acidic protein, S100B, interleukin-1β, and tumor necrosis factor (TNF)-α. *BCHE-K* noncarriers displayed 100%–150% higher glial fibrillary acidic protein and 64%–110% higher S100B than *BCHE-K* carriers, who, in contrast, had 40% –80% higher interleukin-1β and 21%–27% higher TNF-α compared with noncarriers. A high level of CSF BuChE enzymatic phenotype also significantly correlated with higher CSF levels of astroglial markers and several factors of the innate complement system, but lower levels of proinflammatory cytokines. These individuals also displayed beneficial paraclinical and clinical findings, such as high cerebral glucose utilization, low β-amyloid load, and less severe progression of clinical symptoms. In vitro analysis on human astrocytes confirmed the involvement of a regulated BuChE status in the astroglial responses to TNF-α and ACh. Histochemical analysis in a rat model of nerve injury-induced neuroinflammation, showed focal assembly of astroglial cells in proximity of BuChE-immunolabeled sites. In conclusion, these results suggest that BuChE enzymatic activity plays an important role in regulating intrinsic inflammation and activity of cholinoceptive glial cells and that this might be of clinical relevance. The dissociation between astroglial markers and inflammatory cytokines indicates that a proper activation and maintenance of astroglial function is a beneficial response, rather than a disease-driving mechanism. Further studies are needed to explore the therapeutic potential of manipulating BuChE activity or astroglial functional status.

1. Introduction

Over 70 years ago the enzyme butyrylcholinesterase (BuChE) was discovered (Stedman and Easson, 1932), yet as of today, its biological role remains unclear, particularly the essence of BuChE function in the central nervous system (CNS) is largely unknown. However, the distribution pattern and observations in acetylcholinesterase (AChE) knockout mice point at the involvement of BuChE in neural function such as coregulation of cholinergic neurotransmission (Darvesh et al., 2003; Mesulam et al., 2002).

A substantial number of genetic variants of *BCHE* have been identified (Darreh-Shori et al., 2012; Darvesh et al., 2003). The *BCHE-K* variant is the most common functional point mutation of

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this enzyme (Darreh-Shori et al., 2012; Darvesh et al., 2003). Serum BuChE activity of individuals with the *BCHE-K* variant is 30%–33% lower compared with subjects with the wild type variant (Darvesh et al., 2003). Pharmacogenetic studies suggest that the K variant might be involved in the pathogenesis of Alzheimer's disease (AD), particularly in relation to the major known genetic risk factor of AD (i.e., the ε4 allele of apolipoprotein E [ApoE]; Darreh-Shori et al., 2012; Darvesh et al., 2003; Lane et al., 2008). In patients with AD, the difference in the plasma BuChE activity is more pronounced; thus, K heterozygotes show 30% and homozygotes 50% lower BuChE activity than the wild type carriers (Darreh-Shori et al., 2012). The expression of BuChE is also substantially increased in the hippocampus and temporal cortex of patients with AD, whereas expression of AChE is reduced (Perry et al., 1978). A recent postmortem study shows a strong correlation between BuChE activity and L-deprenyl as a measure of activated astroglial cells in the AD brain (Kadir et al., 2011).

The major source of BuChE in the CNS is attributed to nonexcitable cells such as astrocytes and microglia, which also express various nicotinic acetylcholine (ACh) receptors, in particular, the α7 subtype (Carnevale et al., 2007; Shytle et al., 2004), indicating that BuChE might play a regulatory role in the functional status of cholinoceptive nonexcitable cells, such as astrocytes, oligodendrocytes, and microglial cells, via its ACh hydrolyzing activity (Darreh-Shori et al., 2011a). Astroglial cells serve multiple functions in the CNS, including maintenance of the extracellular microenvironment and regulation of neurotransmitter levels (Maragakis and Rothstein, 2006).

Deprenyl binding to monoamineoxidase-B is putatively regarded as a promising positron emission tomography (PET) marker for in vivo assessment of activated astroglial cells in the brain (Carter et al., 2012). Recent in vivo studies with ^{11}C-deuterium-L-deprenyl-PET suggest that presence of activated astrocytes and microglia at early and at late stages of AD (Carter et al., 2012).

Two commonly used cerebrospinal fluid (CSF) glial markers are glial fibrillary acidic protein (GFAP) and S100B. GFAP is the subunit protein of intermediate filaments in astroglial cells. S100B is regarded as an astrocytic cytokine and might mediate interactions between glial cells or between glial cells and neurons (Mrak and Griffin, 2001). However, GFAP is more specific for astrocytes than S100B, because oligodendrocytes, ependymal cells, choroid plexus epithelium, vascular endothelial cells, and some neurons also express S100B in the brain (Steiner et al., 2007).

Hypertrophy of astrocytes accompanied by elevated expression of GFAP is observed in postmortem AD brain with increasing Braak stage (Beach and McGeer, 1988; Kashon et al., 2004; Wharton et al., 2009), and at a given Braak stage, Apo ε4 carriers show slightly higher GFAP levels than noncarriers (Wharton et al., 2009). CSF levels of GFAP also show an age-dependent increase (Rosengren et al., 1994) and an inverse association is reported between cognitive function and GFAP levels in the occipital, parietal, and temporal lobes (Kashon et al., 2004).

Involvement of the innate immune complement (C) system in AD pathology is well established (Veerhuis, 2011) and CSF levels of C3 have recently been reported to correlate with cognitive decline in patients with AD (Wang et al., 2011). However, any relation between BuChE and CSF complement levels has been unknown.

The aim of the current study was to study the relation between a genetic variation in BuChE activity and a number of CSF biomarkers reflecting astroglial activation and innate inflammation. To this end, 179 AD patients were genotyped with regard to *BCHE-K* and ApoE ε4 allele variants, and with proteomic and enzymatic analyses of CSF protein levels of ApoE protein, S100B, and GFAP, interleukin (IL)-1β, and tumor necrosis factor (TNF)-α, and activity and protein levels of BuChE and AChE in CSF and plasma. In addition, several complement components were measured in a subcohort of the AD patients. Then, we challenged some of the findings in vitro using human primary astrocytes.

The finding of a strong association between the K variant allele or ACh-hydrolyzing activity of BuChE and the CSF levels of the glial markers suggests that the BuChE genotype and phenotype exerts regulatory effects on the activity of S100B- and/or GFAP-expressing cells and ongoing pathology or symptoms in AD, possibly via regulation of extracellular ACh levels.

2. Methods

2.1. Patients and CSF and plasma samples

The current study population and the selection criteria are the same as reported recently (Darreh-Shori et al., 2012). Briefly, the AD patients were selected based on the following criteria: for genotyping analysis, all the patients had to have a clinical diagnosis of probable AD, from which at least 1 Mini Mental State Examination (MMSE) assessment was available before treatment with any cholinesterase inhibitors (ChEIs). For the enzymatic and proteomic analysis, in addition, at least 1 CSF or plasma sample (or both) had to be available before any ChEIs therapy. In total, 179 patients fulfilled these criteria. All CSF and plasma samples used in the current study were taken from baseline (i.e., before the ChEIs therapies).

This study and the primary clinical studies were approved by the Ethics Committee of Karolinska University Hospital Huddinge, and the Faculty of Medicine and Radiation Hazard Ethics Committee of Uppsala University Hospital, Uppsala, Sweden. This study was conducted according to the Declaration of Helsinki and subsequent revisions. Informed consent was obtained from each patient or the responsible caregivers.

2.2. Neuropsychological assessments

Global cognitive function was assessed using the standard MMSE test and was available from all of the patients included in the study (Darreh-Shori et al., 2012).

A battery of neuropsychological tests data addressing different cognitive domains (episodic memory, attention/executive ability, and visuospatial ability; Darreh-Shori et al., 2011a) were available from a subgroup of the patients ($n = 66$). To reduce the number of statistical analyses, these data were Z-transformed and the overall composite *Z*-scores of the 3 cognitive domains were calculated as described in Darreh-Shori et al. (2011a).

2.3. PET assessments using fluorodeoxyglucose and Pittsburgh Compound-B

Part of the analysis is based on data acquired in CSF of the patients with probable AD, who had undergone the (18)F-fluorodeoxyglucose (FDG)-PET ($n = 50$) and/or Pittsburgh Compound-B (PIB)-PET ($n = 29$) assessment as described in Darreh-Shori et al., (2011a).

2.4. Single nucleotide polymorphism analysis

The *BCHE* rs1803274 single-nucleotide polymorphism (SNP) (CATATTTACAGG AAATATTGATGAA[A/G]CAGAATGGGA GTGGA-AAGCAGGATT) was used, which corresponds to the so-called K variant of BuChE. The SNP was investigated in genomic DNA using polymerase chain reaction (PCR) using a TaqMan SNP genotyping assay kit according to the manufacturer's instruction and on a StepOne Plus thermal cycler (Applied Biosystems), as described in Darreh-Shori et al. (2012).