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Research article

Association between butyrylcholinesterase and cerebrospinal fluid biomarkers in Alzheimer's disease patients

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HIGHLIGHTS

- 217 AD patients and 200 controls were genotyped for ApoE and BuChE-K variant.
- CSF BuChE activity, Aβ42, t-tau and p-tau levels were determined in 88 AD patients.
- BuChE-K did not seem to confer risk for AD or influence BuChE activity in CSF.
- CSF BuChE activity correlated with Aβ42 levels, particularly in AD ApoE-ε4 carriers.

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ABSTRACT

The deficit of cholinergic activity is one of the main findings in Alzheimer's disease (AD), and is related to the synthesis of acetylcholine, and the hydrolysing enzymes, acetylcholinesterase and butyrylcholinesterase (BuChE). Together with the Apolipoprotein E-ε4 allele (ApoE-ε4), the BuChE-K variant has been proposed to increase AD risk in certain populations. In addition, this polymorphism has been associated with a lower capacity to attenuate β-amyloid aggregation.

In the present study we explored the interaction of the BuChE-K variant with its activity in CSF, conventional AD biomarkers and ApoE genotype.

217 AD patients and 200 age-matched controls were genotyped for the ApoE and the BuChE-K variant. BuChE activity in CSF, as well as the levels of the CSF-AD biomarkers amyloid-beta 42 (Aβ42), total and hyperphosphorylated tau (t-tau and p-tau) were determined in 88 of these patients.

The results showed no significant differences in the BuChE-K variant distribution between patients and controls. No influence of the BuChE-K variant was seen neither in CSF BuChE activity, nor in the levels of Aβ42, t-tau and p-tau in AD patients. ApoE genotype also did not seem to influence CSF BuChE activity. Interestingly, in AD patients, an association between high CSF BuChE activity and increased levels of CSF Aβ42 was shown, particularly in ApoE-ε4 allele carriers.

In our population, the BuChE-K variant does not seem to confer risk for AD or to influence the activity of the enzyme in CSF. However, we demonstrated an association between BuChE activity, ApoE-ε4 genotype and CSF Aβ42 levels, highlighting the importance of assessing BuChE activity as a possible modulator of Aβ load in the brain.

1. Introduction

Alzheimer's disease (AD) is the most prevalent cause of dementia with very high socio-economic burden worldwide [4]. Although AD is mainly a sporadic age-related multi-factorial disorder, with female gender and (low) education as additional epidemiological

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risk factors, genetic contributions are also well-established. Several pathogenic mutations have been identified in familial early-onset forms (EOAD) and ApoE-ε4 allele is a well established risk factor for sporadic early and late-onset AD (LOAD) [11].

Clinical diagnosis of AD is still uncertain and histological confirmation is mandatory for a definitive diagnosis. The main histological hallmarks of AD are amyloid plaques, neurofibrillary tangles and loss of cholinergic neurons and synapses in selective regions of the brain. Amyloid and neurofibrillary pathology can be assessed through the quantitative determination of the 42 amino acid isoform of amyloid-beta (Aβ42), total tau (t-tau) and hyperphosphorylated tau (p-tau) in the cerebrospinal fluid (CSF). These biomarkers have shown a high sensitivity and specificity for discriminating between established AD and other forms of dementia and also to identify AD before onset of dementia. Therefore, these biomarkers have been recently incorporated into the new proposed revised criteria for AD [20,34].

The marked loss of cholinergic neurons, mainly from the basal forebrain, is associated with a deficit in cholinergic activity, that affects not only the acetylcholine receptor subunits [37], but also the acetylcholine-synthesizing enzyme, choline acetyltransferase, and the hydrolyzing enzymes: acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Due to the critical role of acetylcholine in cognitive function [35], most of the licensed pharmacological treatments for AD, are cholinesterase inhibitors (ChEIs). However, these have a modest effect in cognition improvement and not every patient with AD benefits from ChEIs treatment as its optimal duration and long-term efficacy is still unclear.

Butyrylcholinesterase is expressed in most human tissues, and although its activity is increased in the brain of AD patients [36], measurements in CSF and in the synaptic regions are in strong contradiction, showing reduced levels of the enzyme [3,29]. Therefore, the hypothesis that the amount of circulating BuChE in the CSF of AD patients is inversely associated with its trapping in Aβ aggregates has been raised [13,15]. In fact, it has been shown that AD risk factors, such as ApoE-ε4, female gender and advancing age, influence the levels of BuChE in the CSF of AD patients [13,15], and that BuChE interacts in vitro with Aβ peptides and tau-protein [19,41].

BuChE-K variant, an alanine-to-threonine substitution in the 539 amino acid position (Ala539Thr), is one of the most common polymorphisms in the *BuChE* gene. Many studies have been conducted to evaluate the association between BuChE-K variant and AD risk. Recently, in a meta-analysis involving 3850 cases and 3947 controls, a significant association between BuChE-K variant and AD risk in Asians was found, but this result was not replicated in Caucasians [44]. Interestingly, the BuChE-K variant has been associated with a 30% reduction of serum BuChE activity [8]. Moreover, this Ala539Thr substitution is located at the C terminus, which is essential both for BuChE tetramerization and for its capacity to attenuate β-amyloid (Aβ) fibril formation [19,42]. A reduction of the BuChE activity in CSF of AD patients carrying both the K variant and the ApoE-ε4 allele has also been found [15]. However, the influence of the BuChE-K variant in the levels of Aβ42, t-tau or p-tau in the CSF is still unknown.

Therefore, the main aim of this work was to study the relationship between the BuChE-K variant with the activity of the enzyme in CSF. We also wanted to assess the possible interaction between the K variant and the BuChE activity with the conventional CSF-AD biomarkers (Aβ, t-tau and p-tau) and ApoE genotype.

2. Methods

2.1. Study population

The study group consisted of 217 unrelated AD patients and 200 age-matched controls.

AD patients were recruited at the Dementia Clinic, Neurology Department of Coimbra University Hospital. All patients were in a stable condition, without acute comorbidities and underwent a thorough biochemical, neurological and imaging (CT or MRI and SPECT) evaluation. PET, and genetic studies were more restricted, although considered in younger patients. A comprehensive diagnostic battery was administered, including both brief cognitive instruments as the Minimental-State Evaluation (MMSE) [23]. Portuguese version [24], extensive neuropsychological evaluation exploring memory and other cognitive domains and standard staging scales which provide objective information about subject performance in various domains, as previously described [5,6]. All the available information (baseline cognitive test, staging scales, clinical laboratory and imaging studies) was used to reach a consensus research diagnosis. Patients were followed for a minimum of 2 years, after which the clinical diagnosis was revised.

Dementia was diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders – fourth edition (DSM-IV-TR) criteria [2] and AD, according to the National Institute of Neurological and Communicative Disorders and Stroke- Alzheimer's Disease and Related Disorders (NINCDS-ADRDA) [33] and more recently to McKhann et al. [34]. These cases were classified as probable AD according to clinical and neuroimaging features.

The control group was composed of volunteers over 55 years old, recruited from a Portuguese populational study of aging [38], who underwent cognitive evaluation and showed no signs of cognitive impairment.

This study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments and has been approved by the Ethics Board of the Faculty of Medicine of the Universiy of Coimbra and all subjects or responsible caregivers, whichever appropriate, gave their informed consent.

2.2. Laboratory determinations

2.2.1. Sample collection

Peripheral blood samples from all patients and controls were collected into EDTA tubes and genomic DNA was isolated from leucocytes using the DNA isolation kit for mammalian blood (Roche Diagnostics, GmbH, Manheim, Germany), as described by the manufacturer.

From the 217 AD patients included in this study, 105 were submitted to a lumbar puncture, as part of their routine clinical diagnosis investigation, and cerebrospinal fluid samples were collected. At the time of lumbar puncture, 25 patients were already medicated with cholinesterase inhibitors (21 with donepezil and 4 with galantamine). No patients undergoing treatment with rivastigmine were included in the study.

2.2.2. Evaluation of CSF-AD biomarkers

Pre-analytical and analytical procedures were done in accordance with the Alzheimer's Association guidelines for CSF biomarker determination [32]. Briefly, samples were collected into sterile polypropylene tubes, centrifuged at 1800 g for 10 min at 4 °C, aliquoted into polypropylene tubes and stored at −80 °C until analysis. CSF Aβ42, t-tau and p-tau-181 levels were measured by commercially available sandwich ELISA kits (Innotest, Fujirebio, Ghent, Belgium), as previously described [7]. External quality control of the assays was performed under the scope of the Alzheimer's Association Quality Control Program for CSF Biomarkers [32].

2.2.3. Butyrylcholinesterase of CSF activity

The CSF activity of BuChE was measured in 88 AD patients by the method of Ellman [21], with 5,5' Dithio-bis 2Nitrobenzoic ácid (DTNB), butyrylthiocholine iodide as substrate and Ethopropazine