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Mitochondrial DNA differentiates Alzheimer from Creutzfeldt-Jakob disease

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Abstract

Introduction: Low content of cell-free mitochondrial DNA (mtDNA) in cerebrospinal fluid (CSF) is a biomarker of early stage Alzheimer's disease (AD), but whether mtDNA is altered in a rapid neurodegenerative dementia such as Creutzfeldt-Jakob disease is unknown.

Methods: CSF mtDNA was measured using digital PCR in two independent cohorts comprising a total of 112 patients diagnosed with sporadic Creutzfeldt-Jakob disease (sCJD), probable AD, or non-Alzheimer's type dementia.

Results: Patients with AD exhibit low mtDNA content in CSF compared with patients diagnosed with sCJD or with non-Alzheimer's type dementias. The CSF concentration of mtDNA does not correlate with Aβ, t-tau, p-tau, and 14-3-3 protein levels in CSF.

Discussion: Low-CSF mtDNA is not a consequence of brain damage and allows the differential diagnosis of AD from sCJD and other dementias. These results support the hypothesis that mtDNA in CSF is a pathophysiological biomarker of AD.

Keywords: Mitochondrial DNA; Cerebrospinal fluid; Creutzfeldt-Jakob disease; Alzheimer's disease; Biomarker; Digital PCR

1. Introduction

Neurocognitive disorders encompass a heterogeneous group of neurodegenerative diseases, including Alzheimer's disease (AD), Prion disease, Frontotemporal lobar degeneration, and Lewy body disease, which all have in common a decline in brain function or dementia [1]. The differential diagnosis of these diseases is difficult, particularly in early disease stages, because of the marked similarity of clinical signs. Presently, in a clinical setting and for some of these disorders, a definitive diagnosis of the disease that underlies the dementia symptoms can only be established post-mortem after neuropathologic examination. Alternatively, to reach an in vivo diagnosis, which in diseases such as Alzheimer's may be qualified with different levels of probability, current diagnostic guidelines use a combination of clinical phenotype criteria together with the presence of biomarker evidence associated with the particular disease pathology [2,3]. Nonetheless, the time course of the clinical symptoms of dementia differs markedly among neurodegenerative disorders, and some of them exhibit a long preclinical asymptomatic phase. Therefore, differential diagnosis of neurodegenerative disease

CSIC and CIBERNED, institutions where P.P. and R.T. are affiliated, filed a patent application PCT/ES2013/070713 for the diagnosis or prognosis of neurodegenerative diseases.

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subtypes must rely on the association of each subtype with the presence of a particular biomarker profile.

Pathophysiological, core, or diagnostic biomarkers are those hypothesized to be etiologic indicators of the disease, whereas disease progression markers comprise structural or metabolic brain imaging, and biomarkers thought to be a consequence of cell damage [2,4]. Generally, initial detection of neurodegenerative diseases at the preclinical stage should be based preferably on the measurement of pathophysiological biomarkers because disease progression markers may not be present at the early stages of disease. However, with the exception of dominant pathogenic gene mutations, preclinical diagnosis of neurodegenerative diseases with the use of pathophysiological biomarker evidence is still in the process of validation because currently known cerebrospinal fluid (CSF) biomarkers of neurocognitive disorders are also altered in vascular dementia, stroke, and in normal aging [5,6], preventing a precise differential diagnosis. Moreover, the relationship between the appearance of pathophysiological biomarkers and the onset of clinical symptoms of dementia remains poorly understood and under intensive investigation. Furthermore, owing to their association with advanced age, neurodegenerative diseases exhibit a high prevalence of multimorbidity [7] that hinders the detection of the primary disease mechanisms underlying the dementia symptoms. Thus, for a precise differential diagnosis of disorder subtypes, it is necessary to identify disease-specific pathophysiological biomarkers that precede the appearance of disease progression surrogate markers.

Recently, we observed that low content of circulating cell-free mitochondrial DNA (mtDNA) in CSF is associated with both sporadic and familial forms of AD but not with frontotemporal lobe degeneration (FTLD) and found that mtDNA content in CSF differentiates with high sensitivity and specificity AD from FTLD [8]. Moreover, low mtDNA content in CSF occurs in asymptomatic carriers of pathogenic AD mutations at least 1 decade before the expected emergence of clinical signs of dementia and well before any alteration in currently known AD biomarkers in CSF, indicating that low mtDNA is an early biomarker of AD [8]. However, the relationship between mtDNA content in CSF and surrogate markers of brain damage in rapid dementias has not been explored.

In comparison with other neurocognitive disorders, in rapid progressive dementias, such as sporadic Creutzfeldt-Jakob disease (sCJD), dementia symptoms generally exhibit a rapid time course, with an average duration of 4 months in a range of 1–18 months [9] and are associated with marked structural brain lesions [10]. At present, one of the CSF biomarkers most frequently used for the diagnosis of sCJD is the protein 14-3-3 [11]. This protein is released by damaged neurons and is an early surrogate marker of neurodegeneration [12]. Studies in experimental models of dementia evoked by infection with the simian immunodeficiency virus have shown that 14-3-3 protein appears in CSF before the emergence of dementia symptoms and that the appearance of 14-3-3 protein reflects presynaptic neuronal damage, indicating that this protein reveals the early neuronal injury that leads to neurocognitive deficits [13].

We have now studied the relationship between the concentration of mtDNA in CSF and the presence or absence of the prion biomarker 14-3-3, along with $A\beta_{1-42}$, t-tau, and p-tau biomarker levels in patients with sCJD to assess the influence of brain damage on CSF mtDNA and to determine whether the CSF concentration of mtDNA may discriminate AD from rapid progressive dementia.

2. Methods

2.1. Subjects

The study cohort consisted of a total of 44 subjects recruited at Polish neurologic and psychiatric hospital departments (Table 1). Subjects were classified based on clinical profile and CSF levels of t-tau and p-tau in three groups following current recommendations on research diagnostic criteria of AD [2,14]: (1) controls with non-AD dementia: subjects with dementia but with t-tau values <400 pg/mL and p-tau values <50 pg/mL; (2) possible AD: subjects with mild cognitive impairment or dementia with t-tau values >400 pg/mL and p-tau values >50 pg/mL; and (3) sCJD: subjects with a definite diagnosis of sCJD. Neuropathologic diagnosis of prion disease was established post-mortem according to criteria published by Parchi et al. [15] using immunohistochemistry with anti-PrP antibody (clone 12F10, 1:1000, formic acid pretreatment; Cayman Chemical Company) in formalin fixed paraffin embedded tissue.

The validation cohort consisted of a total of 68 subjects recruited at the National Reference Center for Transmissible Spongiform Encephalopathies of the Department of Neurology and Neuropathology at the University Medical Center of Göttingen, Germany (Table 1). All these subjects underwent clinical and neuropsychological assessment and lumbar puncture. The subjects of this cohort were classified in four different groups: (1) neurologic disorder controls: subjects diagnosed with a neurologic disorder without dementia; (2) sCJD: patients classified as definite cases by neuropathologic examinations or as probable sCJD cases according to diagnostic consensus criteria [10,16]; (3) familial Creutzfeldt-Jakob disease (gCJD): subjects carrying the missense mutation E200K (A to G transition at codon 200 with substitution of lysine [K] for glutamate [E]) in the prion protein gene that causes CJD; and (4) AD: patients with diagnosis based on the *International Classification of Diseases, Tenth Revision* definition for AD (F.00-G.30). Cognitive function was assessed in a subgroup of 15 of these AD patients with the Mini-Mental State Examination (MMSE; extracted from CERAD, 0–30 pts) scale.

2.2. CSF samples

Lumbar puncture was performed for diagnostic purposes with analysis of CSF standard parameters at the time point