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Featured Article

Cerebrospinal fluid levels of the synaptic protein neurogranin correlates with cognitive decline in prodromal Alzheimer's disease

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Abstract

Introduction: Synaptic dysfunction is an early event in Alzheimer's disease (AD) pathogenesis and directly related to cognitive impairment. Consequently, synaptic biomarkers may be valuable tools for both early diagnosis and disease stage. Neurogranin (Ng) is a postsynaptic protein involved in memory consolidation.

Methods: We developed three monoclonal anti-Ng antibodies. Mass spectrometry and a novel enzyme-linked immunosorbent assay were used to analyze cerebrospinal fluid (CSF) Ng in three independent clinical cohorts including patients with AD dementia (n = 100 in total), mild cognitive impairment patients (MCI), (n = 40) and controls (n = 80 in total).

Results: We show in three independent clinical cohorts a marked increase in CSF Ng levels in AD dementia ($P < .001$ in all studies). In addition, high CSF Ng levels at the MCI stage predicted progression to dementia due to AD with a hazard ratio of 12.8 (95% confidence interval 1.6–103.0, $P = .02$). In amyloid-positive MCI patients, high CSF Ng correlated with a more rapid change in cognition during clinical follow-up ($P = .03$).

Discussion: These results suggest that CSF Ng is a novel AD biomarker that may be used to monitor synaptic degeneration, and correlates with the rate of cognitive decline in prodromal AD.

Keywords: Neurogranin; Alzheimer's disease; Mass spectrometry; ELISA; Mild cognitive impairment; Prognostic marker

1. Introduction

In the Alzheimer's disease (AD)-affected brain, neuropathology reveals widespread deposits of plaques consisting of amyloid beta (Aβ) peptides and neurofibrillary tangles consisting of phosphorylated tau (p-tau) protein [1]. These changes are reflected in the cerebrospinal fluid

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(CSF) by lower levels of the 42 amino acid long Aβ peptide $A\beta_{1-42}$ together with increased levels of total tau (t-tau) and p-tau [2]. The central hypothesis for AD pathogenesis posits that different forms of Aβ aggregates are neurotoxic, causing synaptic dysfunction and later on progressive neurodegeneration, clinical symptoms, and dementia [3].

Synapses are the functional units in neuronal communication, and synaptic dysfunction is likely directly linked to cognitive disturbance and has long been appreciated as an early and central pathogenic mechanism in AD [4]. The dysfunction of synapses is believed to occur before neuronal degeneration and death [4–6], and synaptic loss seems to be more strongly correlated with cognitive decline than either plaque or tangle pathology [5,7–9]. Both neurogranin (Ng) mRNA and protein levels show an age-related decrease with aging in several brain regions, including the hippocampus [10]. A marked synaptic loss in the hippocampus is present already in the earlier stage of the disease, where synapse numbers correlate with degree of memory dysfunction [8,11]. Thus, fluid biomarkers for synaptic dysfunction would be desirable, because they may be useful for early diagnosis, as tools to monitor synaptic dysfunction during disease progression [9], and to identify drug effects on synaptic function and degeneration in clinical trials. At present, however, such biomarkers are not available.

Neurogranin (Ng) is a postsynaptic protein involved in the regulation of of calmodulin levels in response to increased intracellular calcium after neuronal excitation [12,13]. It plays a critical role in long-term potentiation [14,15] and may also be associated with cognitive function [10]. In the normal human brain, Ng expression is highest in associative cortical areas [16].

In the AD-affected brain, we and others have shown that Ng levels are markedly lower in the hippocampus and the frontal cortex, while no change is seen in the cerebellum [17,18]. In the late 1990s, we showed that a set of synaptic proteins, including Ng, is present in CSF [19]. To evaluate CSF Ng as an AD biomarker, we performed a pilot study using immunoprecipitation to enrich Ng from CSF followed by semiquantitative immunoblotting, and showed an increase in CSF Ng in AD compared with controls [20]. In this assay, we used the NM2 monoclonal antibody, directed against the protein kinase C (PKC) phosphorylation site amino acid sequence QASFR, which is common between neuromodulin (GAP-43) and Ng [21]. Indeed, preabsorption of CSF with the NM2 monoclonal antibody (mAb), increased the sensitivity of the assay to identify Ng [20]. Because the analytical sensitivity of this pilot assay was suboptimal, needing 1.5 ml of CSF [20], due to the lack of monoclonals specific for Ng, we initiated a project to produce novel anti-Ng mAbs with the aim to develop an immunoassay that could be applied in CSF biomarker studies on larger cohorts of patients and controls.

The immunoaffinity purification of Ng from CSF using these novel antibodies followed by mass spectrometric (MS) analysis revealed that Ng is metabolized into a series of C-terminally located peptide species. With this knowledge in hand, we developed a novel ELISA method using anti-Ng antibodies directed to the C-terminal part of Ng which allows the quantification of these Ng peptides in CSF. To test the hypothesis that Ng can be used as a biomarker for AD, reflecting the ongoing synaptic pathology, we analyzed CSF Ng levels in three independent cohorts consisting of patients diagnosed with AD, mild cognitive impairment (MCI), and controls. Here we demonstrate a robust increase of CSF Ng in AD and MCI due to AD (MCI-AD) compared with cognitively normal controls or cognitively stable MCI (sMCI).

2. Methods

2.1. Studies 1 and 2

Demographic and biomarker characteristics of the patients included in the two clinical studies are shown in Table 1. Patients were derived from two different centers specialized in the evaluation of memory disorders. Subjects included in Study 1 were recruited from the Alzheimer Disease Research Center at the Karolinska Institute, Stockholm, Sweden and subjects included in Study 2 were recruited from the Department of Geriatrics, Uppsala University Hospital, Uppsala, Sweden. See Supplementary methods for details on the diagnostic procedures.

2.2. Study 3—validation study

Demographic and biomarker characteristics of the patients included in the clinical study are shown in Tables 1 and 2. Patients included in Study 3 were derived from the memory clinic based Amsterdam Dementia Cohort [22], and selected based on the availability of baseline CSF, which was collected according to international consensus guidelines [23]. Forty patients with a diagnosis of probable AD were matched for age and sex to 40 controls and 40 patients with MCI. See Supplementary methods for details on the diagnostic procedures.

All three studies were conducted according to the provisions of the Helsinki Declaration. All subjects gave written informed consent for the use of their clinical data for research purposes, and the local Ethical Committees at the respective university approved each study.

Pooled de-identified CSF samples collected in routine workflow at the Neurochemistry Laboratory at Sahlgrenska University Hospital, Mölndal, Sweden, were used for method development, as approved by the Ethical Committee at University of Gothenburg.

All CSF samples included in the three studies were collected in polypropylene tubes, centrifuged (1800 g, 10 minutes, +4 °C) and the collected supernatant was stored at −80 °C pending biochemical analysis.