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Research Report

Neurogranin in cerebrospinal fluid as a marker of synaptic degeneration in Alzheimer's disease

Annika Thorsell[a,*], *Maria Bjerke*[a], *Johan Gobom*[a], *Eva Brunhage*[a], *Eugeen Vanmechelen*[b], *Niels Andreasen*[c], *Oskar Hansson*[d], *Lennart Minthon*[d], *Henrik Zetterberg*[a], *Kaj Blennow*[a]

[a]*Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, The Sahlgrenska Academy at University of Gothenburg, Mölndal, Sweden*

[b]*Innogenetics NV, Ghent, Belgium*

[c]*Department of Neurobiology, Caring Sciences and Society, Karolinska Institute, Memory clinic, M51, Department of Geriatric Medicine, Karolinska University Hospital, Huddinge, Stockholm, Sweden*

[d]*Clinical Memory Research Unit, Department of Clinical Science Malmö, University of Lund, Lund, Sweden*

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ABSTRACT

Synaptic pathology occurs early in Alzheimer's disease (AD) development, and cerebrospinal fluid biomarkers for synaptic damage may be altered early in the disease process. In the present study we examined cerebrospinal fluid levels of the postsynaptic protein neurogranin in patients with mild cognitive impairment (MCI) or AD and controls. The low neurogranin level in cerebrospinal fluid required enrichment by immunoprecipitation prior to mass spectrometric identification and semi-quantitative immunoblot analysis. Relative quantification revealed a significant increase of neurogranin in the AD group compared with controls, while the MCI group was not statistically different from either controls or the AD group. The concentrations of the AD biomarkers T-tau, P-tau$_{181}$ and Aβ_{42} were significantly changed in the control and MCI groups compared with the AD group, but no significant differences were found between the MCI group and controls for the three biomarkers. Nevertheless, a trend towards increasing levels of neurogranin, T-tau and P-tau$_{181}$ was found in cerebrospinal fluid from MCI patients compared with controls. The elevated neurogranin levels in the MCI and AD groups might reflect synaptic degeneration. These results together suggest that cerebrospinal fluid neurogranin might be valuable together with the established AD biomarkers in the early diagnosis of AD and warrants further studies to determine the diagnostic value of neurogranin.

* *Corresponding author*. Clinical Neurochemistry Laboratory, Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, The Sahlgrenska Academy at University of Gothenburg, Sahlgrenska University Hospital, Mölndal, SE-431 80 Mölndal, Sweden. Fax: +46 31 343 2426.

E-mail address: annika.thorsell@neuro.gu.se (A. Thorsell).

Abbreviations: AD, Alzheimer's disease; CSF, cerebrospinal fluid; IP, Immunoprecipitation; MCI, mild cognitive impairment; MS, mass spectrometry

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1. Introduction

One of the most important and challenging clinical tasks in the field of Alzheimer's disease (AD) is making diagnosis in an early stage of the disease. The identification of sensitive and specific biomarkers for AD may enable early diagnosis and may help to evaluate the efficacy of potential therapeutic interventions. Synaptic pathology occurs early in AD development suggesting that alterations in the axonal or synaptic compartment are a primary event in the progression of the disease (Masliah et al., 1994; Standridge, 2006; Stokin and Goldstein, 2006; Terry, 1996). Further, synaptic pathology is strongly correlated with cognitive impairment (DeKosky and Scheff, 1990; DeKosky et al., 1996; Honer, 2003). Hence, biomarkers for synaptic activity could be good indicators of early synaptic dysfunction and might prove valuable for early diagnosis. Since cerebrospinal fluid (CSF) is in direct contact with the central nervous system and contains proteins and metabolites that reflect brain biochemistry, potentially promising biomarkers might be found in the CSF.

In the present study we used methods for immunoprecipitation (IP) of neurogranin in CSF compatible with either mass spectrometric identification or semi-quantitative immunoblotting analysis to determine whether neurogranin could serve as an early diagnostic biomarker for AD. Neurogranin is the dendritic analogue of presynaptic neuromodulin in the postsynaptic membrane (Watson et al., 1994). It binds to calmodulin at low concentrations of Ca2+ and is involved in activity-dependent synaptic plasticity in neurons and in long-term potentiation (LTP) important for learning and memory through modulation of Ca2+/calmodulin-dependent pathways (Gerendasy, 1999; Gerendasy and Sutcliffe, 1997; Huang et al., 2004; Klann et al., 1992; Xia and Storm, 2005). Moreover, reduced neurogranin levels were found in brain specimens from AD patients compared with those from controls (Davidsson and Blennow, 1998; Reddy et al., 2005). This is to our knowledge the first study in which the neurogranin levels in CSF from patients with mild cognitive impairment (MCI), AD and controls have been studied. Additionally, neurogranin was identified in CSF by mass spectrometry (MS).

2. Result

2.1. *Mass spectrometric identification of neurogranin in CSF*

An IP method compatible with MS-analysis was developed with the aim to identify neurogranin in CSF. The capture antibody was chemically cross-linked to the beads to avoid co-elution of the antibody into the immunoprecipitated sample. Further, in order to evaluate the potential loss of binding strength as a consequence of cross-linking, experiments were performed in which the capture antibody was immobilized non-covalently to the beads as well as covalently cross-linked. The performance of the IP was monitored by immunoblotting analysis revealing that the antibody can be cross-linked without any significant loss of capture efficiency (Lanes 4 and 5 of Fig. 1). Elution was performed with an aqueous formic acid solution compatible with downstream MS-analysis and its elution capacity was investigated by performing a second elution with the more stringent NuPAGE® sample buffer. The second elution verified that the elution capacity of the acidic solution was satisfactory. Immunoprecipitated samples from the first elution resulted in intense neurogranin bands (Lanes 4 and 5 of Fig. 1), while they were notably reduced in samples from the second elution (Lanes 6 and 7 of Fig. 1). Moreover, the neurogranin capture antibody also has specificity for neuromodulin in the CSF (Oestreicher et al., 1994). With aim to improve the recovery of neurogranin further and to investigate the effect of neuromodulin, the CSF was depleted of neuromodulin prior to neurogranin IP. The antibody employed in the depletion by IP has specificity for neuromodulin but not neurogranin. Lanes 8 and 9 of Fig. 1 show that IP performed with a combination of both antibodies improved the neurogranin recovery as a result of the depletion of neuromodulin that otherwise competes with neurogranin. Depleted CSF was immunoprecipitated and the resulting sample was subjected to tryptic digestion followed by MS analysis. Neurogranin was identified in the database search of MS/MS data confirming that the immunoprecipitated sample contains neurogranin (Fig. 2). Neurogranin is a 7.6 kDa peptide that generates only five tryptic peptides with at least five amino acids. Three peptides were identified within the 95% significance interval in the database search of MS/MS spectra. The sequence coverage was 26% of the protein. Although neuromodulin and neurogranin share some sequence homology, two peptides unequivocally could be identified as being derived from neurogranin, thus confirming its presence (Fig. 2). The combined approach of IP and MS/MS therefore allowed, for the first time, identification of neurogranin by MS in CSF.

2.2. *Study of neurogranin CSF levels by immunoblotting*

The mass spectrometric identification of neurogranin in the immunoprecipitated samples warranted subsequent quantitative analysis of neurogranin in CSF. An IP approach optimized for semi-quantitative immunoblotting analysis was employed, since the low neurogranin concentration in CSF does not allow immunoblot analysis without enrichment (Lane 2 of Fig. 1). The anti-rabbit neurogranin antibody recognized a band in the IP samples, immunoprecipitated by the anti-mouse neurogranin NM2 antibody, on the immunoblots with an apparent molecular weight identical to the recombinant protein (Fig. 1). The use of the different antibodies increased the verification of the identity of neurogranin while decreasing the IP capture IgG interference on the immunoblots.

2.2.1. *Study I*

The neurogranin levels in CSF from 11 AD patients and 9 controls were compared in Study (I). The neurogranin level, assessed by the semi-quantitative immunoblotting analysis, was found to be significantly increased ($p<0.005$) in AD patients compared with controls (Fig. 3A). The T-tau ($p<0.005$) and P-tau$_{181}$ ($p<0.005$) concentration was significantly increased in AD patients, while the Aβ_{42} ($p<0.01$) concentration was significantly decreased compared with controls (result not shown). The neurogranin levels in the CSF were then tested for association with the biomarker concentrations. The neurogranin levels were positively correlated with T-tau ($\rho=0.75$, $p=0.01$, Fig. 4A) and P-tau$_{181}$ ($\rho=0.73$, $p=0.01$) in the AD group, while no correlation was