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Featured Article

Synaptic proteins predict cognitive decline in Alzheimer's disease and Lewy body dementia

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Abstract

Introduction: Our objective was to compare the levels of three synaptic proteins involved in different steps of the synaptic transmission: Rab3A, SNAP25, and neurogranin, in three common forms of dementia: Alzheimer's disease (AD), dementia with Lewy bodies (DLB), and Parkinson's disease dementia.

Methods: A total of 129 postmortem human brain samples were analyzed in brain regional specific manner exploring their associations with morphologic changes and cognitive decline.

Results: We have observed robust changes reflecting synaptic dysfunction in all studied dementia groups. There were significant associations between the rate of cognitive decline and decreased levels of Rab3 in DLB in the inferior parietal lobe and SNAP25 in AD in the prefrontal cortex. Of particular note, synaptic proteins significantly discriminated between dementia cases and controls with over 90% sensitivity and specificity.

Discussion: Our findings suggest that the proposition that synaptic markers can predict cognitive decline in AD, should be extended to Lewy body diseases.

Keywords: Dementia with Lewy bodies; Alzheimer's disease; Parkinson's disease with dementia; Cognitive impairment; Synaptic dysfunction; SNAP25; Rab3A; Neurogranin

1. Introduction

The pandemic increase in the number of people with dementia carries serious implications for society [1–5]. Although there has been a tremendous increase in research and efforts to develop new treatments, this has largely focused on Alzheimer's disease (AD). The synuclein dementias, DLB, and Parkinson's disease dementia (PDD), present with a particularly challenging constellation of symptoms and account for 15% of people with dementia but have received far less attention. As in AD, cholinesterase inhibitors provide symptomatic benefits, but efforts to develop disease-modifying therapies are at a much earlier stage. Previous pathologic studies have suggested that the burden of synuclein pathology is associated with cognitive

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decline, and that concurrent AD pathology may also contribute [6]. However, this only explains a minority of the variance, and a better understanding of disease substrates is needed for targeted drug discovery and to enable better monitoring of disease progression. The structural basis of dementia in most neurodegenerative disorders is considered to be neuronal and synaptic loss accompanied by intraneuronal protein aggregation [7]. Changes in synaptic function are usually reflected by alterations in the concentration of synaptic proteins in the presynaptic or at the postsynaptic density [8]. A significant decrease in cortical synapses has been reported in AD [9,10]. Importantly, initial work suggests that the loss of synapses is more robustly correlated with cognitive decline in these individuals than traditional markers of AD pathology [11], suggesting that these changes are already evident at the earliest stages of disease [12]. Less is known regarding the role of synaptic dysfunction in PDD and DLB [6,13,14], but synaptic alterations have been demonstrated in Parkinson's disease [15], and preliminary studies have indicated early synaptic changes in DLB/PDD. Consistent with our hypothesis that synaptic dysfunction may be particularly important in DLB/PDD, structural imaging studies indicate that brain atrophy is less pronounced in DLB and PDD compared to AD [16] despite the more severe disease course [17,18]. Synaptic dysfunction has been also suggested to be caused by presynaptic accumulation of alpha-synuclein aggregates [19].

The aim of the current work was therefore to investigate the importance of synaptic changes in DLB/PDD and AD and to provide a more detailed characterization of synaptic changes to inform further drug and biomarker discovery. We focused our attention on three synaptic proteins that on the grounds of their differential role in the synaptic machinery represent high-priority candidates for investigation.

Neurogranin is one of the main postsynaptic proteins involved in the regulation of synaptic transmission through its binding to calmodulin at low levels of calcium [20]. Synaptosomal-associated protein 25 (SNAP25) is known to provide the driving force for vesicle fusion and docking [21]. The presynaptic vesicle protein, Rab3A, reflects the recycling pool of synaptic vesicles [22].

In the present study, we used an exploratory approach to examine brain regional specific distribution of these three synaptic proteins, on prospectively followed, clinically and neuropathologically well-characterized patients with DLB, PDD, AD, and controls without dementia. Such information may aid in the development of new diagnostic and prognostic biomarkers as well as novel mechanism-based treatments.

2. Materials and methods

2.1. Brain tissue

Postmortem human brain tissue (from 129 cases in total) as well as brain sections (17–19 section/brain region) were provided by the Brains for Dementia Research network including cases from the Newcastle Brain Tissue Resource (21 cases), the Thomas Willis Oxford Brain Collections (17 cases), and the London Neurodegenerative Diseases Brain Bank (65 cases) as well as from the University Hospital Stavanger (26 cases). Autopsy protocols and sample collection were harmonized among all the centers. Samples from four different brain regions including prefrontal cortex (BA9), temporal lobe neocortex (BA21), anterior cingulate cortex (BA24), and inferior parietal lobe neocortex (BA40) were studied.

In total, 34 PDD patients (age 68–89 years), 52 DLB patients (age 65–92 years), 18 AD patients (age 72–103 years), and 25 aged non-neurological controls (age 65–96 years) were included. Not all patients had tissues available for all brain regions and analyses. Assessment and diagnostic criteria have been previously described [6].

Cognitive impairment data were available for most of the patients (Supplementary Table 1) and consisted of the last mini-mental state examination (MMSE) scores, assessed usually within 1–2 years before death [23] as well as of MMSE decline calculated as the decline per year averaged over the period of clinical observation consisting of generally 8–10 years. All participants gave informed consent for their tissue to be used in research, and the study was approved by the UK National Research Ethics Service (08/H1010/4), the Norwegian committee for medical and health research ethics (2010/633), and by the Regional Ethical Review Board in Stockholm (2012/920-31/4).

2.2. Preparation of tissue samples

Preparation of tissue for western blotting and ELISA analyses was performed as previously described and can be found in more details in supplementary material [2].

2.3. Sandwich enzyme-linked immunosorbent assays

We have developed sandwich ELISA for each of the studied synaptic proteins. With the exception of the antibodies, the method was identical regardless of the antigen. Details regarding antibodies and purified proteins are described in Supplementary Table 2. Detailed protocol is described in supplementary methods. Samples of human brain were added in dilutions of 0.1 μg/μL of total protein, and standards were diluted so that the sample absorbance values would fall near 50% binding (the linear range) of the standard curve. The coefficient of variation was $<$20% and the accuracy between 80% and 120% for acceptance. Concentrations were calculated after the mean blank value had been subtracted.

2.4. Immunoblotting

To minimize interblot variability, 20-μg total protein and/or samples were loaded in each lane of each gel on 7.5%–10% SDS-polyacrylamide gel for protein separation and then transferred to nitrocellulose membrane (Immobilon-P, Millipore). Each gel contained a control lane of pooled brain homogenates used as an internal standard. After blocking nonspecific binding, membranes were incubated with primary antibodies followed by HRP-conjugated secondary antibody.