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 (PDD).

METHODS: 129 post-mortem human brain samples were analyzed in brain regional specific manner exploring their associations with morphological 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.

Abbreviations: Rab3A- Ras-related protein Rab-3A, SNAP25- synaptosomal-Associated Protein, 25kDa**,** BA9- prefrontal cortex, BA21- temporal lobe neocortex, BA24- anterior cingulate cortex, BA40 inferior parietal lobe neocortex

1. INTRODUCTION

The pandemic increase in the number of people with dementia carries serious implications for society [1-5]. Whilst there has been a tremendous increase in research and efforts to develop new treatments, this has largely focussed on AD. The synuclein dementias, DLB and 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 pathological studies have suggested that the burden of synuclein pathology is associated with cognitive 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 pre-synaptic or at the post-synaptic 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 is 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 employed 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

Post-mortem 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 was harmonized among all the centres. 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 was available for the majority 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 [2]. Briefly, 500 mg of frozen tissue was homogenized in ice-cold buffer containing 50 mM Tris-HCL, 5 mM EGTA, 10 mM EDTA, protease inhibitor cocktail tablets (Roche, 1 tablet per 50 mL of buffer), and 2 mg/mL pepstatin A dissolved in ethanol:dimethyl sulfoxide 2:1 (Sigma). The buffer was used at a ratio of 2mL to every 100 mg of tissue, and homogenization was performed using an IKA Ultra-Turrax mechanical probe (IKA Werke, Germany) until the liquid appeared homogenous. Protein concentration of each sample was measured by using BCA Protein Assay Kit (Thermo Scientific). Samples

for ELISA measurements were further diluted to 0.1 μg/ μL total protein in phosphate buffer saline (PBS) buffer.

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 less than 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 inter-blot variability, 20 μg total protein/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 non-specific binding, membranes were incubated with primary antibodies followed by HRP conjugated secondary antibody. GAPDH was used as a reference protein assessing equal loading. Equal concentration of pooled brain homogenate sample was used as an internal control loaded on each gel. Bands were visualized using Chemiluminescent substrate (Millipore) in a LAS-3000 luminescent image reader (Fujifilm). Western blot data were evaluated and quantified using Multi Gauge Image Analyzer (version 3.0).

2.5 Immunohistochemistry

Brain tissue was acquired and assessed pathologically as previously described [6]. Formalin fixed, wax-embedded blocks, cut into 7µm sections and mounted onto slides, were used for immunohistochemistry. Briefly, the sections were dewaxed and rehydrated using Histoclear and alcohol dilutions. Antigen retrieval was carried out by microwaving the sections for ten minutes in citrate buffer pH 6.0. Following blocking of endogenous peroxidases (0.3% H_2O_2 in PBS for 30 minutes), sections were incubated overnight with antibodies specific for neurogranin-1, SNAP25 and Rab3A. Development of the sections was performed using biotinylated secondary antibodies, ABC reagents and a DAB kit (all Vector Laboratories, Peterborough, UK). Sections were briefly counterstained with Mayer's hematoxylin solution before dehydration, mounting with DPX and coverslipping. For control experiments, the secondary biotinylated antibody was omitted.

2.6 Statistical analysis

For the univariate descriptive analyses, considering the irregular non-Gaussian distribution of the samples, nonparametric statistical tests were used. To assess the relationship between synaptic proteins neuropathological and MMSE scores, Spearman correlations were performed. To compare protein levels between controls and the different patient groups we used Kruskal-Wallis tests, followed by Dunn`s post hoc test using the IBM SPSS Statistics 22 software. In all cases differences were considered statistically significant when $p \leq 0.05$ (*), $p \leq 0.01$ (**) or $p \leq 0.001$ (***).

Multivariate data analyses was performed to assess the ability of synaptic proteins (when combined together) to discriminate controls from the different patient groups using orthogonal partial least square analyses (SIMCA, version 13.0; Umetrics AB, Sweden). Detailed description of the multivariate statistical analysis can be found in the supplementary methods section. We calculated the sensitivity, specificity, positive predictive values and negative predictive values of the group separations from the $Q^2(Y)$ values obtained in each

model. Results from the multivariate analyses were visualized in loadings plots, where the synaptic proteins are presented on the x-axis according to their importance for the separation of the different groups. Measures below zero have a lower value in patients whereas measures above zero have a larger value in patients compared to the control group. Covariance is plotted on the y-axis. For every synaptic protein, the Jack-knifed confidence intervals are shown. The proteins with a confidence interval that includes zero have low classification power.

3. RESULTS

3.1 Demographical characteristics of the samples

Key cohort characteristics are shown in Table 1. There were no significant differences in the pH or in the post-mortem delay between the groups. AD patients were significantly older than all the other three groups but no other group differed significantly in age. Correlations between age and MMSE decline scores were observed in PDD in the prefrontal cortex (Rho=0.553, p=0.0076, n=22), temporal lobe (Rho=0.369, p=0.0346, n=33) and in the inferior parietal lobe (Rho=0.369, p=0.0346, n=33).

3.2 Differences in the levels of synaptic proteins between diagnostic groups

Specificity of the antibody pairs employed in the ELISA studies assessed by immunoprecipitation in cerebrospinal fluid show that all the 3 pairs of antibodies were highly specific for recognizing neurogranin, Rab3A or SNAP25 proteins (Supplementary Figure 1). Western blot and ELISA analyses both demonstrated that, both pre-synaptic proteins Rab3A and SNAP25 and postsynaptic protein neurogranin had reduced levels across the brain regions in the three dementia groups compared to controls (Table 2, Figure 1). We have observed an overall 20-51% decrease in the levels of synaptic proteins in DLB patients, 16-

41% in PDD patients and 22-42% decrease in AD patients compared to non-neurological controls throughout all the four studied brain regions. ELISA assay indicated similar changes in most brain regions.

Immunohistochemistry supported the direction of changes seen for neurogranin in most of the examined cortical areas with only moderate decrease identified for Rab3A and no overall differences found for SNAP25 (Supplementary Figure 2). Figure 1A- 1H illustrates typical immunolabeling of presynaptic proteins in the anterior cingulate cortex (BA24) and Figure 1I- 1L displays typical immunolabeling of neurogranin in the temporal lobe (BA21) in non-neurological controls versus the various dementia cases.

3.3 Panel of pre and postsynaptic proteins discriminate between control and dementia diagnoses

The multivariate analyses results showed that synaptic protein levels were able to provide a clear separation between controls and the different patient groups. All models were statistically significant and showed sensitivity, specificity, positive and negative predictive values that were all above 90% in the case of PDD and DLB groups and were close to 90% in AD patients (Table 3).

The first model showed a good predictive power of $Q^2(Y) = 0.699$ in discriminating controls from patients with PDD. The variables that contributed to the separation between these groups were SNAP25 in BA9 and BA24, Rab3A in BA40 and BA21, neurogranin in BA40, BA24 and BA21 (Fig. 2). The second model showed a sound predictive power of $Q^2(Y) = 0.719$ in discriminating controls from DLB patients. All synaptic proteins significantly contributed to the separation between groups, with the exception of Rab3A in BA24 and SNAP25 in BA21 (Fig. 2). The third model showed a good predictive power of $Q^2(Y) = 0.781$ in the discrimination of controls from AD patients. The synaptic proteins that significantly contributed to the separation were neurogranin in BA40, BA9 and BA24,

SNAP25 in BA9 and BA24, and Rab3A in BA40 and BA9 (Fig. 2). Significant separation was not achieved between the three neurodegenerative diseases (data not shown).

3.4 Associations between synaptic proteins and neuropathological scores

Significant associations between synaptic protein levels and neuropathological measures are summarized in Supplementary Table 3 and significant correlations are plotted in Supplementary Figure 3. There were significant correlations between the postsynaptic neurogranin levels and tangle scores in the prefrontal cortex of PDD patients (Rho= 0.529, p = 0.009, n=23) as well as in the anterior cingulate cortex (Rho= 0.737 p = 0.001, n=16) and in the inferior temporal lobe (Rho= 0.529, p = 0.029, n=17) of AD patients. Synaptic vesicle protein Rab3A correlated significantly with alpha-synuclein in the inferior parietal lobe both in PDD (Rho= 0.439, p = 0.011, n=32) as well as in DLB (Rho=-0.3, p = 0.045, n=45). Rab3A presented further positive correlations with plaque scores in the temporal lobe (Rho= 0.475, p = 0.025, n=22) in PDD. Presynaptic protein SNAP25 presented negative correlations with tangle scores in AD in the inferior parietal lobe (Rho= -0.696, p = 0.025, n=10).

3.5 Correlations between synaptic proteins and cognitive impairment

Ten out of 44 DLB cases in BA40 and two out of 18 AD cases had no MMSE decline scores available (Supplementary Table 1). We observed that the rate of cognitive decline was associated with both presynaptic proteins, Rab3A and SNAP25. Inverse correlations to MMSE decline were present for Rab3A and SNAP25 levels assessed by ELISA assay in patients with DLB and AD respectively (Fig. 3). Reduced Rab3A and SNAP25 levels were associated with a more rapid cognitive decline in the inferior parietal lobe in the case of Rab3A (Rho=-0.424, p=0.012, n=34) and in prefrontal cortex in the case of SNAP25 (Rho=-0.502, p=0.047, n=16). In addition, we have performed correlations without a potential outlier for SNAP25, and the correlation coefficient barely changes, from -0.502 to -0.499, indicating that the observed association is not driven by this potential outlier. In contrast,

neurogranin showed an association with higher MMSE scores measured at the last assessment prior to death in DLB patients (inferior parietal lobe Rho=-0.388, p = 0.023, n=34) (Supplementary Figure 4). No significant associations were found between synaptic proteins and cognition in Parkinson`s disease.

4. DISCUSSION

Our biochemical studies conducted in post-mortem brain tissues have highlighted significant loss of pre- and post-synaptic proteins in DLB and PDD. The pre- and postsynaptic proteins examined were selected for their roles in crucial synaptic processes such as vesicle docking (SNAP25), vesicle recycling (Rab3A) and postsynaptic signalling (neurogranin). Changes in these proteins were able to accurately discriminate between age matched controls and individuals with PDD and DLB with high sensitivity and specificity (>90%). Synaptic protein changes of Rab3A and neurogranin in the inferior parietal lobe (BA40) as well as SNAP25 in prefrontal cortex (BA9) provided the best discrimination between the disease groups and non-neurological controls. These proteins also merit further evaluation as disease biomarkers and the related synaptic pathways are highlighted as potentially important treatment targets.

Prefrontal and cingulate cortex displayed the most robust synaptic protein changes in all three dementia groups with decrease in both pre- and postsynaptic protein levels between 23-43% compared to control non demented cases. The most robust significant changes reflecting synaptic dysfunction were observed in DLB patients followed by PDD cases and AD patients. A handful of studies have shown synaptic loss in AD [11, 24-26]. A recent study has detected early synaptic dysfunction that preceded amyloid beta pathology in animal model for familial AD (APP/PSEN1), where independent of tau pathology, synaptic dysfunction was already detectable prior to the early rise of different soluble amyloid beta peptides [27]. Synaptic dysfunction has been also shown in Parkinson`s disease [15], whereas

much less is known regarding synaptic pathology in DLB. We have recently identified reductions of synaptophysin, syntaxin and synaptosome-associated protein in the visual association cortex in DLB and an association between zinc transporter 3 and postsynaptic density protein 95 and the level of cognitive impairment in DLB have been reported [28]. More recently, reductions of synaptophysin as well as syntaxin and synaptosome-associated protein of 25 kD (SNAP-25) were identified in the visual association cortex in DLB [29], a brain area where pathological changes are usually not pronounced [30], suggesting the possibility that these may be early pathological changes.

More detailed studies, including other brain regions and a larger panel of proteins are needed to explore whether there are disease-related differences. The current work adds important new information by confirming the discriminatory power of changes in synaptic proteins to distinguish between the respective disease groups and controls, and highlights change in specific synaptic proteins.

Recent studies have suggested that early stages of tau accumulation have a toxic action on synaptic function [31, 32]. Positive correlations between neurogranin and p-tau and total tau in cerebrospinal fluid of patients with AD were reported [33], supporting the notion that cerebrospinal fluid concentrations of neurogranin and possibly other synaptic proteins are directly or indirectly associated with the level of synaptic degeneration. We found that both the postsynaptic protein neurogranin as well as the presynaptic protein SNAP25 were positively correlated with tangle scores in different brain regions. Similar positive associations between another postsynaptic protein, postsynaptic density protein 95 and increasing amyloid beta levels in cortical regions have been reported in Alzheimer's disease, suggesting that certain forms of synaptic process are strongly involved in the regional specificity of both tau and amyloid beta levels [34, 35].

We have found a number of associations with the level of Lewy body pathology. Specifically, the presynaptic Rab3A protein was associated with tangle scores and alpha-synuclein scores in the anterior cingulate cortex and the inferior parietal lobe. Interestingly, the Rab3A recycling machinery is closely linked to synuclein's membrane association and dissociation cycle known to increase α-synuclein sequestration leading to cognitive consequences [36]. Our results presenting associations between Rab3A and synuclein pathology are in line with previous findings reporting increased binding of Rab3A to alpha-synuclein aggregates in DLB [37], proposing that decreased Rab3A levels, is likely to directly affect not only the reserve synaptic vesicle pool but alpha-synuclein pathology as well. The detailed relationship between synaptic machinery proteins with tau, amyloid beta and α-synuclein levels needs to be further explored. It remains possible that some regional associations observed in our study might be mediated by other confounding factors but it definitely underlines the involvement of the synaptic activity in the various dementias.

Among the main findings of this study are that decreased Rab3A and SNAP25 levels correlated with increased rate of cognitive decline in DLB and AD, respectively. In DLB, the region showing such associations for Rab3A was the inferior parietal lobe, which has been associated with the typical visuospatial impairment [38, 39]. Associations for SNAP25 and cognition in AD were limited to the prefrontal cortex. Prefrontal cortex is known to be involved in executive functions and cognition [40], the deterioration of which is encountered early in the development of Lewy body dementias while it is affected only in later stages of AD. The apparent contradiction in the involvement of the synaptic pathology and cognitive association of the affected regions, i.e. parietal lobe in dementia with Lewy body and prefrontal cortex in AD, may represent the implication of different clinical features but also underlines the importance of the disease stage of the sample available for post-mortem studies since we have seen cognitive correlations of synaptic proteins in regions that are

involved late in the course of the diseases. As post-mortem brain studies usually have limited number of early stage pathology, cerebrospinal fluid or serum samples of early dementia patients could add another level to our current knowledge and progression of these dementias. Our current data suggest that stabilization of Rab3A levels may represent an important treatment strategy in DLB patients, while SNAP25 could be a new marker in the progression of AD.

One of the strengths of our study is that we have combined multiple techniques to measure synaptic changes in brain tissues which generally point towards the same conclusions namely the loss of presynaptic and postsynaptic proteins throughout the studied brain regions. Another advantage of our study is the relatively large number of cases with DLB and PDD, giving us more potential to mitigate the influence of the individual changes that occur in smaller cohorts. One limitation of our study is that our AD cohort was comprised of only 18 cases, however our results are in agreement with previous findings regarding SNAP25 as a promising biomarker for synapse degeneration in AD[41]. The availability of longitudinal standardized clinical data in brain bank studies is a relatively unique feature of our study though longitudinal MMSE scores were not available for all patients (Supplementary Table 1). Finally, the prospective design with standardized cognitive assessments is a unique strength of the study.

4.1 Conclusions and future implications

Loss of synapses is an early and robust correlate of cognitive decline in Alzheimer's disease [11, 25], and accumulating evidence point in the direction of the importance of synaptic changes in other dementias as well, including DLB and PDD [8, 28, 42]. Our work strongly supports this as an exciting area, with potential to improve diagnosis and identify novel therapeutic targets. Cortical reductions of three pre- and post-synaptic proteins discriminated Lewy body dementia and AD from non-neurological controls with high sensitivity and

specificity. In addition, we report evidence that decreased levels of presynaptic vesicle protein Rab3A and SNAP25 are associated with cognitive impairment and neuropathological scores in DLB and AD.

Our findings suggest that the proposition that synaptic markers can predict cognitive decline in AD [33], should be extended to Lewy body diseases. Finally, the relationship of Rab3A and SNAP25 to disease pathology and impact on cognitive decline highlights their importance as a treatment target and its potential as a future biomarker of disease progression for clinical trials is a path that should be further explored.

RESEARCH IN CONTEXT

Systematic review: A significant decrease in cortical synapses has been previously reported in AD. Initial work suggests that the loss of synapses is more robustly correlated with cognitive decline than with traditional markers of AD pathology, suggesting that these changes are already evident at the earliest stages of disease.

Interpretation: Decreased Rab3A and SNAP25 levels correlated with increased rate of cognitive decline in DLB and AD and neuropathological markers suggesting that stabilization of synaptic protein levels such as Rab3A may represent an important treatment strategy in DLB patients, while SNAP25 could be a new marker in the progression of AD.

Future directions: The role of synaptic proteins as possible predictors of cognitive decline in AD and DLB needs to be evaluated by further biomarker studies in CSF samples.

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CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

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FIGURE LEGENDS AND TABLES

Figure 1. **Changes in synaptic protein levels assessed by Western blotting and immunolabelling**.
Rab3A (A- D) and SNAP25 (E- H) immunostaining was performed in BA24, while neurogranin
staining (I- L) was performed in BA21. Compared to non-neurological controls (A and I) both Rab3A
and neurogranin immunolabelling revealed mild to moderate decreased levels of synaptic proteins in
PDD (B and J) and DLB (C and K), while no changes were observed in AD cases. SNAP25 showed
similar levels of immunoreactivity in non-neurological controls (E) and the dementia cases studied (F-
H). Scale bars represent 25 microns. Neurogranin, Rab3A and SNAP25 levels present changes in
prefrontal cortex (BA9) assessed by semiquantitative western blotting (Panel M and N). Statistical
analyses were performed using Kruskal-Wallis test followed by post hoc Dunn`s multiple comparison
test. Neurogranin levels both in PDD, DLB and AD were significantly reduced when compared to
non-neurological controls. Similarly, Rab3A levels were significantly reduced in all three dementia
diagnoses when compared to controls. SNAP25 levels were also found to be decreased in PDD and
DLB while they were found to have comparable levels in AD and control non-neurological cases. The
bars represent the mean values with inter-quartile range. Abbreviations used C=control,
NRGN=neurogranin. For all figures * $p<0.05$, **$p<0.01$, ***$p<0.001$

**Figure 2. Multivariate analyses results showing the contribution of synaptic proteins to
discriminate controls from the different patient groups.** Plots showing the variables of importance
and their corresponding jack-knifed confidence intervals for the separation between controls and PDD
patients (A), controls and DLB patients (B), controls and AD patients (C). A measure with high
covariance is more likely to have an impact on group separation than a variable with low covariance.
Measures with confidence intervals that include zero have low reliability.

Figure 3. Correlations between presynaptic proteins and cognitive decline. SNAP25 and Rab3A
protein levels assessed by ELISA analyses present negative correlations with cognitive impairment
assessed by yearly MMSE decline scores in prefrontal cortex (BA9) and inferior parietal lobe (BA40)
of AD patients and DLB patients respectively, analysed by nonparametric Spearman correlations.

Table 1. Demographic characteristics of the subjects used in this study according to clinical diagnosis.

Diagnosis	Gender (M/F) %	Age at death	PMD (h)	pH	MMSE at death	MMSE decline	Coded Braak staging
Control (25)	60/40	79.8 ± 1.5	39.1 ± 4.6	6.47 ± 0.05	n/a	n/a	1 (0-2)
PDD (34)	53/47	79.9 ± 1.0	33.5 ± 2.7	6.44 ± 0.06	12.7 (0-27)	2.1 ± 0.3	1 (0-2)
DLB (52)	60/40	81.3 ± 0.9	41.5 ± 3.9	6.37 ± 0.06	13.9 (0-30)	3.0 ± 0.4	3 (1-3)
AD (18)	33/67	88.1 ± 1.7	35 ± 5.3	6.30 ± 0.08	8.5 (0-19)	3.5 ± 0.9	3 (2-3)

Values are expressed with standard error of the mean, coded Braak stage is presented as the mean with the range in brackets. Coded Braak stages represent neurofibrillary Braak staging 0=0; 1= stages 1/ 2; 2=stages 3/ 4 and 3=stages 5/ 6. Abbreviations used: PMD=post mortem delay, MMSE= mini mental state examination.

Table 2. **Differences in the synaptic proteins neurogranin, Rab3A and SNAP25 assessed by Western blotting and ELISA.**

WB

	NRGN BA9		NRGN BA21		NRGN BA24		NRGN BA40	
PDD	38%	p<.01	30%	p<.05	36%		45%	p<.001
DLB	40%	p<.01	51%	p<.001	35%	p<.01	46%	p<.001
AD	35%	p<.05	NA		24%		28%	

	Rab3A BA9		Rab3A BA21		Rab3A BA24		Rab3A BA40	
PDD	30%	p<.01	16%		43%	p<.05	24%	
DLB	41%	p<.01	27%	p<.05	39%	p<.01	42%	p<.001
AD	42%	p<.01	NA		38%	p<.01	22%	

	SNAP25 BA9		SNAP25 BA21		SNAP25 BA24		SNAP25 BA40	
PDD	37%	p<.05	26%	p<.05	35%	p<.05	33%	p<.001
DLB	42%	p<.01	24%	p<.05	41%	p<.001	20%	
AD	23%		NA		33%	p<.05	34%	p<.01

ELISA

	NRGN BA9		NRGN BA21		NRGN BA24		NRGN BA40	
C	185.4 (83.1)		132.9 (34.8)		158.1 (35)		201.8 (41.2)	
PDD	114.2 (56.1) (38%)	p<.05	115.1 (40) (13%)		123 (13) (22%)	p<.05	130.6 (27.9) (35%)	p<.001
DLB	142.4 (78.4) (23%)		91.3 (22.3) (31%)	p<.001	124.5 (30) (21%)	p<.01	125.9 (34.4) (38%)	p<.001
AD	95.2 (49.6) (49%)	p<.001	NA		141.6 (23.1) (10%)		117.7 (17) (42%)	p<.001

	Rab3A BA9		Rab3A BA21		Rab3A BA24		Rab3A BA40	
C	180.6 (40.9)		152.4 (56.3)		112.6 (28.6)		157.3 (26.9)	
PDD	150.3 (33.3) (17%)		128.8 (39.3) (15%)		113 (20.1) (0%)		103.2 (17.4) (34%)	p<.001
DLB	138 (31.3) (24%)	p<.05	104 (29.3) (32%)	p<.001	106.8 (24.8) (5%)		115 (15.7) (27%)	p<.001
AD	132.7 (25.3) (27%)	p<.01	NA		99.95 (18.78) (11%)		113.2 (14.6) (28%)	p<.001

	SNAP25 BA9		SNAP25 BA21		SNAP25 BA24		SNAP25 BA40	
C	178.7 (35.7)		98.9 (29.5)		158.8 (35.7)		142.3 (30.8)	
PDD	95.3 (27.6) (47%)	p<.001	85.2 (18.9) (14%)		135.1 (24) (15%)	p<.05	84.9 (37.4) (40%)	p<.05
DLB	92.1 (24) (48%)	p<.001	90.9 (20) (8%)		129.2 (33.2) (19%)	p<.05	75.5 (43.9) (47%)	p<.01
AD	107.3 (32.1) (40%)	p<.001	NA		126.7 (28.5) (20%)	p<.05	85.75 (25.5) (40%)	p<.01

Differences in protein levels between disease groups and controls were determined using Kruskal-Wallis test followed by Dunn`s post hoc test. Western blot changes are expressed as percentage compared to control, while ELISA values are expressed in pg/mL (means ± standard deviation) as well as in percentage changes compared to control in parenthesis. Abbreviations used: NRGN = neurogranin. p values represent statistical differences resulting from comparisons of dementia cases to non-demented control groups.

Table 3. Sensitivity, specificity, positive and negative predictive values for each model.
Abbreviations used: C= Control CI= confidence interval; PPV= positive predictive value; NPV= negative predictive value.

Models	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
PDD vs C	97.1 (85.1-99.5)	100 (80.6-100)	100 (89.3-100)	94.1 (71.2-99.0)
DLB vs C	94.7 (82.7-98.5)	93.8 (71.7-98-9)	97.3 (85.8-99.6)	100 (78.0-100)
AD vs C	82.4 (59.0-93.8)	90.5 (77.3-99.2)	87.5 (61.6-98.1)	86.3 (65.1-96.9)
PDD vs DLB	56.8 (40.9-71.3)	63.6 (46.6-77.8)	63.6 (45.1-79.6)	56.8 (39.5-72.9)
PDD vs AD	43.8 (19.8-70.1)	81.8 (64.5-93.0)	53.9 (25.1-80.8)	75.0 (57.8-87.9)
DLB vs AD	43.8 (19.8-70.1)	81.1 (64.8-92.0)	50.0 (23.0-77.0)	76.9 (60.7-88.9)