

1 **Title**

2 CSF neurogranin as a neuronal damage marker in CJD: a comparative study with AD.

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## 38 ABSTRACT

39 **Objective:** To investigate whether cerebrospinal fluid (CSF) neurogranin concentrations are altered in  
40 sporadic Creutzfeldt-Jakob disease (CJD), comparatively with Alzheimer's disease (AD), and  
41 associated with neuronal degeneration in brain tissue.

42 **Methods:** CSF neurogranin, total-tau(tau), neurofilament light(NFL) and 14-3-3 protein were  
43 measured in neurological controls (NC,n=64), (AD (n=46) and CJD (n=81). The accuracy of  
44 neurogranin discriminating the three diagnostic groups was evaluated. Correlations between  
45 neurogranin and neurodegeneration biomarkers, demographic, genetic and clinical data were assessed.  
46 Additionally, neurogranin expression in post-mortem brain tissue was studied.

47 **Results:** Compared to NC, CSF neurogranin concentrations were increased in CJD (4.75 times of NC;  
48  $p<0.001$ , AUC (95%CI)=0.96 (0.93-0.99) and AD (1.94 times of NC;  $p<0.01$ , AUC (95%CI)=0.73  
49 (0.62-0.82), and were able to differentiate CJD from AD ( $p<0.001$ , AUC (95%CI)=0.85 (0.78-0.92)).  
50 CSF tau was increased in CJD (41 times of NC) and in AD (3.1 times of NC), both at  $p<0.001$ . In  
51 CJD, neurogranin positively correlated with tau ( $\rho=0.55$ , $p<0.001$ ) and was higher in 14-3-3-  
52 positivity ( $p<0.05$ ), but showed no association with NFL ( $\rho=0.08$ , $p=0.46$ ). CJD-MM1/MV1 cases  
53 displayed higher neurogranin levels than VV2 cases. Neurogranin was increased at early CJD disease  
54 stages and was a good prognostic marker of survival time in CJD. In brain tissue, neurogranin was  
55 detected in the cytoplasm, membrane and post-synaptic density fractions of neurons, with reduced  
56 levels in AD, and more significantly in CJD, where they correlated with synaptic and axonal markers.  
57 **Conclusions:** Neurogranin is a new biomarker of prion pathogenesis with diagnostic and prognostic  
58 abilities, which reflects the degree of neuronal damage in brain tissue in a CJD subtype manner.

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## 60 Keywords

61 Neurogranin, cerebrospinal fluid; neurodegenerative dementias; Creutzfeldt-Jakob disease,  
62 Alzheimer's disease, tau, neurofilament light.

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## 64 INTRODUCTION

65 Neurogranin is a calmodulin-binding protein abundantly expressed in the soma and dendrites of  
66 neurons of the telencephalon[1,2] involved in synaptic plasticity and long-term potentiation[3,4].  
67 Neurogranin has been suggested to be a specific cerebrospinal fluid (CSF) Alzheimer's disease (AD)  
68 biomarker, since its concentration is increased in AD, but not in other neurodegenerative diseases (*i.e.*,  
69 frontotemporal dementia, Lewy body dementia, Parkinson's disease, progressive supranuclear palsy,  
70 multiple system atrophy and Huntington's disease)[5–7]. Although CSF neurogranin presents only  
71 moderate diagnostic value for AD[5,8], this can be improved when combined with other CSF  
72 biomarkers of AD such as tau and neurofilament light (NFL)[9]. In AD, CSF neurogranin displays  
73 strong positive correlation with other AD biomarkers such as tau and phospho-tau[5,10–13], while

74 weak or no correlations were detected with amyloid-beta42, a biomarker of amyloid plaques  
75 load[5,10,13].

76 A prognostic value for neurogranin in AD has been proposed, as its CSF concentration is  
77 differentially elevated in mild cognitive impairment (MCI) patients with biomarker AD-signature[11]  
78 as well as in MCI patients who progress to AD dementia compared to those who remain cognitively  
79 stable[10,13]. Similarly, CSF neurogranin correlates with rate of cognitive decline in MCI[14] and  
80 with reduction of brain volume in AD[8]. In cognitively normal individuals, CSF neurogranin is also  
81 useful in predicting future cognitive impairment[8]. Regrettably, neurogranin analysis in paired  
82 plasma-CSF samples indicated that the AD-specific increased CSF levels are not reproduced in  
83 plasma, discarding the potential use of blood neurogranin measurements for diagnostic or prognostic  
84 purposes[15].

85 Although extensive work has been done in AD, data is lacking regarding neurogranin levels in other  
86 diseases presenting substantial synaptic and neuronal loss. This is the case of prion diseases, one of  
87 whose fundamental characteristics is synaptic degeneration and disorganization, which leads to  
88 neuronal loss and spongiform changes. Indeed, over a 30% reduction in the relative synaptic index has  
89 been reported in prion disease-affected brains compared to controls[16]. Similarly to AD, synaptic  
90 loss occurs at early stages of prion diseases[17], and it is suggested that synaptic pathology is initiated  
91 at the synaptic spine[18]. Experiments conducted in prion disease mouse models revealed that axon  
92 terminal degeneration and synaptic loss precede neuronal death and are associated with the onset of  
93 clinical symptomatology[19]. Sporadic Creutzfeldt-Jakob disease (CJD) is the most prevalent human  
94 prion disease characterized by rapidly progressive dementia and short disease duration [20]. The  
95 combination of genotype at codon 129 (methionine or valine) and PrPSc type (1 or 2 based on the size  
96 of protease resistant PrP fragments) gives rise to different CJD subtypes with characteristic disease  
97 phenotype and neuropathological features. Thus, synaptic and neuronal damage, neuroinflammation,  
98 deposition of pathogenic prion protein (PrPSc) and lesion profile occur in a well-defined regional- and  
99 subtype-specific manner[17,21–23].The most prevalent subtypes are CJDMM1/CJDMV1 (60-70% of  
100 the cases) with predominant cortical affection and, CJD VV2 (~16% of the cases), with prominent  
101 cerebellar affection [22]. Several pathological mechanisms are suggested to contribute to CJD  
102 synaptic pathology, including the accumulation of the abnormal form of prion protein in synaptic  
103 structures[24].

104 In the present study, we quantified CSF neurogranin in CJD and AD cases in order to comparatively  
105 unveil its diagnostic and prognostic potential. We also characterized the presence of neurogranin in  
106 CJD and AD brains to investigate the underlying pathological conditions in the central nervous  
107 system that may lead to the observed disease-specific CSF signatures.

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## 111 **METHODS**

### 112 **Antibodies**

113 The monoclonal neurogranin antibody Ng2 was produced using KLH-conjugated peptide Ng52–75 as  
114 immunogen, as described previously[14] and was used (1:400) for immunohistochemistry (IHC). The  
115 neurogranin antibody Ng36 was generated using the same protocol, but with KLH-conjugated peptide  
116 Ng63-75 as immunogen and was used for western blot (1:6000). Antibodies against sodium-  
117 potassium adenosine triphosphatase (ATPaseNa/K $\beta$ , Affinity-MA3-930;1:2000), glyceraldehyde3-  
118 phosphate dehydrogenase (GAPDH, Abcam ab9485;1:2500), postsynaptic density protein 95 (PSD-95,  
119 Thermo-Fisher-7E3-1B8;1:1000), synaptophysin (SYNP, Novocastra-NCL-L-SYNAP-299;1:4000),  
120 total-tau (tTau, Sigma-T5530;1:500) and beta-actin ( $\beta$ -actin, Sigma-A5316;1:30000) were used in the  
121 western blot experiments.

### 122 **Patients and CSF sampling**

123 Neurological controls (NC) were composed of patients diagnosed with a neurological or psychiatric  
124 disease non-associated with a primarily neurodegenerative disease, and were diagnosed according to  
125 acknowledged standard neurological clinical and para-clinical findings based on the 10th revision of  
126 the International Statistical Classification of Diseases definitions. NC include the following diagnoses:  
127 alcohol abuse, astrocytoma, bipolar disorder, cerebral lymphoma, cerebral vasculitis, depression,  
128 epilepsy, Graves' disease, acute or chronic headache, acute hypoxia, ischemic stroke, meningitis,  
129 multiple infarct, pain syndromes, paraneoplasia, paranoid psychosis, peripheral polyneuropathy,  
130 psychosis, schizophrenia, vascular encephalopathy, vasculitis and vertigo. AD was diagnosed  
131 according to the National Institute on Aging-Alzheimer's Association workgroups(NIA-AA)  
132 criteria[25]. CJD was diagnosed according to consensus criteria[26], 60 definite and 21 probable CJD  
133 cases were included. All CSF samples were collected at the Clinical Dementia Center and the  
134 National Reference Center for CJD Surveillance in the Department of Neurology of the University  
135 Medical Center of Göttingen, Germany.

136 Lumbar punctures (LPs) were performed for diagnostic purposes at the first evaluation. For disease  
137 stage, samples were stratified in three categories according to whether CSF was collected in the first  
138 (early) (time of LP to disease onset/total duration of the disease < 0.33), second (middle) (0.33–0.66)  
139 or third (last) (> 0.66) stage of the disease. Disease duration was recorded as the time (in months)  
140 from symptom onset to the death of the patient.

### 141 **Brain samples**

142 Brain tissue was obtained from the Institute of Neuropathology HUB-ICO-IDIBELL-Biobank  
143 following the guidelines of Spanish legislation on this matter (Real Decreto de Biobancos 1716/2011).  
144 Control cases had not suffered from neurologic or psychiatric diseases, infections of the nervous  
145 system, brain neoplasms, or systemic and central immune diseases, and did not have abnormalities in  
146 the neuropathological examination. Neurofibrillary tangles stages were categorized according to  
147 Braak and Braak modified for paraffin sections[27]. CJD cases underwent neuropathological

148 diagnosis according to established neuropathological criteria[28]. Information about brain cases used  
149 in this study is detailed in Supplementary Table 1. CSF was not available for study in any of the post-  
150 mortem brain series.

#### 151 **CSF analyses**

152 Neurogranin and NFL were quantified using two in-house enzyme-linked immunosorbent assay  
153 (ELISA) as described before[13,29]. Total-tau (tau) was quantified using the ELISA kit  
154 INNOTEST®hTAU-Ag (Fujirebio Europe, Ghent, Belgium). CSF was analyzed for the presence of  
155 14-3-3 protein by Western blot according to established CJD diagnostic protocol[30]. The analysts  
156 were blinded to clinical data.

#### 157 **Immunohistochemistry**

158 De-waxed sections, 4 micrometer thick, were processed for immunohistochemistry and incubated at  
159 4°C overnight with one of the primary antibodies and then incubated with R.T.U. Biotinylated  
160 Universal Antibody (Vector,BP1400) for 30 min at room temperature followed by R.T.U. HRP-  
161 Streptavidin (Vector,SA-5704). The peroxidase reaction was visualized with diaminobenzidine and  
162 hydrogen peroxidase. Control of the immunostaining included omission of the primary antibody.  
163 Immunostaining of neurogranin levels were quantified using Image J software, using thresholding tool  
164 settings to subtract background and allow quantification of neuronal neurogranin.

#### 165 **Brain homogenates, subcellular fractionation and western blot.**

166 The purification of PSD fractions from human post-mortem brain tissue was performed as published  
167 before[31]. Brain homogenates and fractions were mixed with SDS-PAGE sample buffer, boiled, and  
168 subjected to 8-15% SDS-PAGE. Gels were transferred onto nitrocellulose membranes and probed for  
169 specific immunodetection by chemiluminescence (ECL-Amersham) using the indicated antibodies.  
170 Densitometries were carried out with the ImageJ software and for brain homogenates values were  
171 normalized using  $\beta$ -actin or GAPDH levels. Since Neurogranin was expressed in all subcellular  
172 fractions, difference among NC, AD and CJD cases was determined in the input. Brain homogenates  
173 were mixed with NuPAGE (Thermo-Fisher) LDS buffer and Reducing Agent, boiled and subjected to  
174 electrophoresis in NuPAGE Bis-Tris 4-12% gels (Thermo-Fisher). Proteins were transferred to  
175 polyvinylidene difluoride (PVDF) membranes and immunodetection was performed as mention above.  
176 Densitometries were determined with the ImageJ software and were normalized using  $\beta$ -actin levels.

#### 177 **Statistical tests**

178 According to distributional features, Mann-Whitney U tests or unpaired t-tests were used to compare  
179 two groups of samples; Kruskal-Wallis test followed by Dunn's post-hoc tests or ANOVA test  
180 followed by Tukey's post-hoc tests was applied for multiple comparisons. To assess the diagnostic  
181 accuracy of neurogranin in the discrimination of the diagnostic groups, receiver operating  
182 characteristic (ROC) curve analyses were carried out and areas under the curve (AUC) with 95%  
183 confidence intervals (95%CI) were calculated using GraphPad-Prism6.01. The best cut-off values  
184 were estimated based on the Youden index. Spearman rank and Pearson correlation coefficients were

185 used to assess associations between continuous biomarker levels. Comparison between AUC was  
186 performed using the DeLong's test[32], available in the R package pROC[33]. To determine the  
187 association between neurogranin, NFL and tau concentrations and total disease duration we used a  
188 fractional polynomial approach based on linear regression methodology as provided in the Stata  
189 package "mfp". The prognostic capacity of potential biomarkers was assessed using Somers' D,  
190 Harrell's C (the higher the better the prognosis) and Brier Scores at 12 months (the lower the score,  
191 the better the prognosis) based on Cox regression models.

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## 193 **RESULTS**

### 194 **CSF neurogranin in AD and CJD**

195 The study population included NC (n=64), AD (n=46) and CJD (n=81) cases. CSF NFL showed a  
196 mild increase in AD (1.3 times of NC;p<0.05) and a marked increase in CJD (4.3 times of  
197 NC;p<0.001). CSF tau showed a moderate increase in AD (3.1 times of NC; p<0.001) while levels in  
198 CJD were very markedly (41 times) higher than in NC (p<0.001). Additionally, increased tau and  
199 NFL concentrations were detected in CJD compared to AD (p<0.001) (Figure 1A) in agreement with  
200 previous studies [34,35].

201 Highest neurogranin concentrations were detected in CJD (571±291 pg/mL), followed by AD  
202 (233±191pg/mL) and NC (120±65pg/mL) (Figure 1A). Neurogranin was significantly different in NC  
203 vs. AD (p<0.01), NC vs. CJD (p<0.001) and AD vs. CJD (p<0.001) (Figure 1B). To determine the  
204 diagnostic accuracy of neurogranin in discriminating the three diagnostic groups, pairwise AUCs were  
205 calculated. Neurogranin poorly discriminated AD from NC (AUC=0.73, 95%CI=0.62-0.82), but  
206 displayed high accuracies distinguishing CJD from NC (AUC=0.96, 95%CI=0.93-0.99) and CJD  
207 from AD (AUC=0.85, 95%CI=0.78-0.92) (Figure 1C). In agreement to this, pROC analysis for the  
208 comparison of AUC values indicate that the AUC for the NC vs CJD comparison was significantly  
209 higher than the AUC for the NC vs AD (p<0.001). A cut-off of 285 pg/mL revealed 89% sensitivity  
210 and 92% specificity for the discrimination of CJD from NC in the study population. In comparison,  
211 diagnostic accuracy of 14-3-3 and tau in the discrimination of CJD from NC was 89% sensitivity/95%  
212 specificity (14-3-3) and 91% sensitivity/98% specificity (tau).

213 The diagnostic value of neurogranin in the discrimination of CJD from NC (AUC=0.96) was  
214 statistically lower than the one for tau (AUC=0.99, 95%CI=0.97-1, pROC neurogranin vs tau,  
215 p=0.012), but higher than the one for NFL (AUC=0.89, 95%CI=0.83-0.95, pROC neurogranin vs  
216 NFL p=0.041).

217 The diagnostic value of neurogranin in the discrimination of CJD from AD (AUC=0.85) was lower  
218 than the one for tau (AUC=0.94, 95% CI=0.91-0.99, pROC neurogranin vs tau, p=0.001) and not  
219 significantly different than the one for NFL (AUC=0.84, 95%CI=0.76-0.91, pROC neurogranin vs  
220 NFL, p=0.84).

221 Next, we compared the accuracy of neurogranin in the discrimination of CJD from rapidly progressive  
222 AD(rpAD), which turns to be challenging in clinical scenario. AD cases with available data on disease  
223 duration (n=32) were stratified in those with disease survival shorter (rpAD, n=11) and longer (AD,  
224 n=21) than 2 years following the definition of Grau-Rivera et al. for rapidly progressive dementia [36].  
225 Neurogranin concentrations were higher in rpAD (256pg/mL) than in AD (214pg/mL), but those were  
226 not significantly different (p=0.47). Similarly, neurogranin was not significantly different for the CJD  
227 vs. AD (p<0.001) and CJD vs. rpAD (p<0.001) comparisons.

### 228 **Influence of demographic and genetic parameters on neurogranin concentrations**

229 Neurogranin concentrations in CJD were neither affected by age at LP (ranging from 43 to 90 years  
230 old, rho=0.05, p=0.64) (Figure 2A) nor by the sex of the patients (p=0.80) (Figure 2B). Similarly, no  
231 strong associations between neurogranin and age at LP and sex were detected in NC (age at  
232 LP:p=0.27, sex:p=0.16), and AD (age at LP:p=0.18, sex:p=0.77) (Figure 2A and Figure 2B). To test  
233 whether genetic characteristics of the patients were associated with differential neurogranin  
234 concentrations, we stratified CJD samples by prion protein gene (*PRNP*) codon 129 genotype (data  
235 available for 65 cases), a well-known CJD risk factor and disease modifier[37]. Mean neurogranin  
236 concentrations were significantly lower in valine/valine [VV] (n=14, 384±172pg/mL) compared to  
237 methionine/methionine [MM] (n=38, 630±318pg/mL) and methionine/valine [MV] (n=13,  
238 640±249pg/mL) cases (p<0.05) (Figure 2C). To explore whether neurogranin was associated with  
239 prion disease subtype, we further stratified CJD cases with known prion subtype achieved through  
240 post-mortem brain tissue analysis (n=28). CJD MM1/MV1 (n=15) and VV2 (n=8) cases, representing  
241 the two most prevalent CJD subtypes were studied. Due to their low number, other subtypes were not  
242 included in the analysis. Neurogranin concentrations were significantly higher in CJD MM1/MV1  
243 (718±306 pg/mL) compared to CJD VV2 (373±160 pg/mL) (p<0.01) (Figure 2D).

### 244 **Correlations between neurogranin, surrogate prion biomarkers and clinical data**

245 In CJD, CSF neurogranin showed a good correlation with tau (rho=0.55, p<0.001), but did not  
246 correlate with NFL (rho=0.08, p=0.46) (Figure 3A). Additionally, tau and NFL displayed a positive  
247 but weak correlation (rho=0.26, p=0.01), in agreement with previous reports[34]. CJD cases  
248 displaying positive 14-3-3 test presented higher neurogranin levels than those showing no 14-3-3 (or  
249 traces) signal in the western blot test (p<0.05) (Figure 3B).

250 To study a potential association between neurogranin levels at the time of lumbar puncture and the  
251 timeliness of the disease in CJD patients, samples were stratified in early, middle and late stages.  
252 Neurogranin concentrations were not significantly different between early (n=9, 510±292 pg/mL),  
253 middle (n=26, 576±294 pg/mL) or late (n=28, 635±319 pg/mL) disease stages (Figure 3C).

254 Next we assessed the potential role of neurogranin as a biochemical marker of disease survival in 63  
255 CJD cases where disease duration was available, and compared it with the performance of tau and  
256 NFL. When allowing for non-linear associations between biomarker levels and disease duration,  
257 neurogranin was able to explain more of the variability in disease duration (R<sup>2</sup>=0.19) than tau

258 ( $R^2=0.10$ ) and NFL ( $R^2=0.07$ ). All three biomarkers showed a log-linear decrease with increasing  
259 disease duration (Figure 3E for neurogranin). For neurogranin, the association with survival time can  
260 be modelled using a linear combination of the terms: neurogranin (in g/ml) =  $533 + 1/(47 * [\text{survival}$   
261  $\text{time in months} - 1.6]) - 28 * [\text{survival time in months} - 0.6]$ ; it showed a good ability as a prognostic  
262 marker, represented by Somers' D value of 0.32; Harrell's C value of 0.66 and a Brier score at 12  
263 months of 0.09. For tau and NFL, similar values were achieved (tau: Somers' D=0.27, Brier  
264 score=0.11; NFL: Somers' D=0.16, Brier score=0.09). In AD, total disease duration was available in  
265 32 cases, in which neurogranin values were also associated with disease (as well via a log-linear  
266 decline,  $R^2=0.32$ ).

### 267 **Neurogranin expression in brain tissue**

268 In human brain tissue of control cases, neurogranin was highly expressed in the neuronal soma of the  
269 cerebral cortex (n=13) and hippocampus (n=6), but absent in the white matter (n=13) and cerebellum  
270 (n=8) (Figure 4A). To further study neurogranin subcellular levels, different brain fractions from  
271 control cases (n=4) were purified. Neurogranin was detected in the cytoplasmic ( $41 \pm 5\%$ ), membrane  
272 ( $32 \pm 4\%$ ) and post-synaptic density (PSD) ( $27 \pm 2\%$ ) fractions. As control proteins for each fraction  
273 we used PSD-95 (post-synaptic), ATPase Na/K $\beta$  (plasma membrane) and synaptophysin (pre-synaptic)  
274 for membrane fraction and GAPDH (cytoplasm) (Figure 4B).

275 Neuronal neurogranin levels were analyzed in the cerebral cortex (control, n=10, AD, n=10, CJD, n=9)  
276 and hippocampus (control, n=6, AD, n=7, CJD, n=5) (Figure 5A). A multiple-comparative tests analysis  
277 of neurogranin expression from immunohistochemical analysis revealed a significant decrease in CJD  
278 ( $p < 0.001$ ) and AD ( $p < 0.001$ ) compared to controls in both brain regions (Figure 5B). Additionally,  
279 neurogranin immunostaining in CJD was significantly lower than in AD in both brain regions ( $p < 0.01$   
280 in cerebral cortex and  $p < 0.05$  in hippocampus). No statistical differences were detected in neurogranin  
281 levels between Braak stages IV (n=3), V (n=4) and VI (n=3), indicating that alterations in neurogranin  
282 expression were not an end-stage feature on AD pathology (Figure 5A).

283 Reduction of neurogranin levels in the frontal cortex of CJD MM1 (n=10) and VV2 (n=10) cases  
284 compared to controls (n=8) was validated by western blot analysis and accompanied by decreased  
285 levels of post-synaptic (PSD-95), pre-synaptic (synaptophysin) and axonal (tau) markers (Figure 6A  
286 and 6B). Compared to controls, and similar to PSD-95, synaptophysin and tau, decreased neurogranin  
287 levels were more severe in CJD MM1 ( $p < 0.001$ ) than VV2 cases ( $p < 0.05$ ) (Figure 6B). Neurogranin  
288 in CJD (n=20) correlated significantly with tau and PSD-95 ( $p < 0.001$ ) and with synaptophysin  
289 ( $p = 0.01$ ). All four proteins presented close correlations with each other (Figure 6C).

290 Neurogranin levels by means of western blot analysis in the frontal cortex region of AD cases (n=18)  
291 were also reduced significantly compared to controls (n=23,  $p < 0.01$ ). Moderate decreases in synaptic  
292 proteins PSD-95 ( $p < 0.01$ ) and synaptophysin ( $p < 0.01$ ) were detected, while tau levels were not  
293 altered (Figure 7A and 7B). Neurogranin in AD (n=18) significantly correlated with synaptophysin  
294 ( $p < 0.001$ ) and PSD-95 ( $p < 0.05$ ) but not with tau ( $p > 0.05$ ). An additional correlation was detected



295 between PSD-95 and synaptophysin ( $p=0.01$ ) (Figure 7C). No significant associations between age,  
296 sex, post-mortem time delay and neurogranin levels measured by western-blot were found in controls,  
297 CJD and AD cases.

298

## 299 **DISCUSSION**

300 In this study, we demonstrate that CSF neurogranin is increased in CJD compared to NC (4.75 fold  
301 change) and AD (2.5 fold change), reaching good diagnostic accuracies in the discrimination of CJD  
302 from AD (AUC=0.85, 95% CI=0.78-0.92). The increased CSF neurogranin concentrations detected in  
303 CJD compared to AD is in line with the lower neurogranin levels detected in the cerebral cortex and  
304 hippocampus of CJD cases, and with the well-known higher neuronal damage present in CJD  
305 compared to AD.

306 In CJD, CSF neurogranin concentrations at early disease stages were not different from those detected  
307 at middle and late stages, indicating that synaptic damage is an early event in CJD, similar to what  
308 previously has been found for AD[8]. Indeed, the observation that neurogranin levels in AD brain  
309 tissue were not different between early and late Braak stages further supporting that synaptic loss, as  
310 measured by neurogranin, is not a late stage pathological event. In this regard, it is well known that  
311 synaptic damage is an early event in AD [38].

312 In our study population, CSF neurogranin correlated neither with age nor with sex in any of the  
313 diagnostic groups but we detected differences in CJD cases regarding codon 129*PRNP* polymorphism  
314 and subtype with potential clinical implications. First, neurogranin concentrations were significantly  
315 higher in CJD MM and MV compared to VV cases, in contrast to tau, which shows higher  
316 concentrations in MM and VV, compared to MV cases [39]. Since codon 129*PRNP* data are pre-  
317 mortem available, the combined analysis of tau and neurogranin could led to specific codon 129*PRNP*  
318 polymorphism-dependent cut-offs enhancing the discriminatory value of single biomarker  
319 measurements. Second, CJD MM1/MV1 cases, two subtypes with similar clinico-pathological  
320 phenotype, displayed higher CSF neurogranin concentrations than VV2. As described before[21] and  
321 in the present study, synaptic and neuroaxonal damage is higher in CJD MM1/MV1 than in VV2 in  
322 cortical regions, where neurogranin is highly expressed. Thus, it is tempting to speculate that CSF  
323 neurogranin levels reflect the neuropathological heterogeneity of CJD prion subtypes regarding  
324 synaptic and neuronal loss. In this regard, biomarkers such as neurogranin, able to recapitulate the  
325 heterogeneity of CJD pathology, may turn into valuable markers for disease diagnosis, prognosis and  
326 for, monitoring potential therapeutic approaches and inclusion of patient populations in clinical trials.  
327 Limitations of this study were the low number of CJD cases with subtype available and the absence of  
328 CSF-brain paired cases. Thus, further analysis including less prevalent subtypes and paired cases  
329 should be carried out to determine the complete neurogranin profile in the spectrum of CJD cases and  
330 its association with neuropathological correlates.

331 Compared to 14-3-3, one of the gold standards CSF biomarkers for CJD, neurogranin presented  
332 similar diagnostic accuracies in the discrimination of CJD from controls. In contrast, tau showed a  
333 much more fold change (41 times as compared with 4.75 times for neurogranin) and higher diagnostic  
334 accuracy than neurogranin in the discrimination of CJD cases from NC and AD. However,  
335 neurogranin explained more of the variance in disease duration than tau and NFL. Further studies  
336 should clarify the precise value of neurogranin over tau and other described prognostic markers for  
337 CJD[34,40] and its precise context of use in disease monitoring and evaluation of eventual therapeutic  
338 therapies. Similarly, in the AD cases, neurogranin was also associated with disease survival,  
339 validating previous reports in which neurogranin was proposed as a marker of AD outcome[8,41].

340 An interesting finding from our study is the observation that neurogranin is broadly present in  
341 different neuronal fractions/compartments. Immunohistochemical analysis was supported by  
342 biochemical studies where we detected similar neurogranin levels in the cytoplasmic, membrane and  
343 post-synaptic fractions. The fact that only a percentage (27%) of total neurogranin is expressed in the  
344 post-synaptic fraction calls attention to its proposed use as post-synaptic damage marker, and suggests  
345 a dual role as a synaptic and neuroaxonal damage marker.

346 Our studies in brain tissue also indicated a major overlap between neurogranin and tau expressing  
347 neurons in the cerebral cortex (data not shown), which explains the high degree of association  
348 between both proteins in the CSF of CJD cases, where major neuronal damage occurs. Likewise, the  
349 absence of a clear correlation between CSF neurogranin and NFL in CJD can be explained by the lack  
350 of overlap between the levels of both proteins in the brain tissue. In this regard, NFL expression is  
351 mainly reported in the axons of the white mater region[42] where neurogranin staining was  
352 undetectable in our cases. Additionally, these results are in agreement with the recent observation that  
353 NFL in the CSF, in contrast to neurogranin, is more increased in CJD VV2 cases than in MM1[34],  
354 with VV2 cases showing higher subcortical pathology compared with other CJD subtypes[43]. Indeed,  
355 neurogranin paralleled the CJD subtype-dependent reduced expression levels of PSD-95,  
356 synaptophysin and tau, showing a significant correlation with all the studied proteins, especially with  
357 tau and PSD-95. Whether these associations are relevant for the neurodegenerative process in CJD  
358 remains unknown due to the rapid and massive synaptic and neuronal damage occurring in this  
359 pathology. In contrast, reduction of synaptic markers was only moderate in AD brain, while tau levels  
360 were unchanged, most likely due to its aggregation in the brain tissue. Moderate decline on synaptic  
361 markers in AD tissue observed in our study was not surprising. While synaptophysin was reported to  
362 be decreased ( $\approx 25\%$ ) in the cortex of mild AD patients[44], recent studies revealed only a moderate  
363 decline in synaptic markers, including PSD-95 and synaptophysin in the prefrontal cortex (BA9) of  
364 patients with AD at advanced cognitive deterioration[45].

365 Similar to CJD, neurogranin levels in AD correlated with both synaptic markers. On the one hand, this  
366 indicates that neurogranin, while not specifically expressed in synapsis, but rather in several neuronal  
367 compartments, could be a synaptic dysfunction marker in AD and CJD. On the other hand, our data

368 also suggest that both pre and post-synaptic dysfunction can be surveyed through the evaluation of  
369 biological fluids. In this regard, it would be interesting to determine whether novel biomarkers that  
370 may be more specific to the synapse[46–48] are differentially altered in AD and CJD and better  
371 reflect synaptic damage than neurogranin.

372 Recently, the presence of increased neurogranin processing peptides and decreased full-length protein  
373 has been reported in AD brain tissue[49]. These observations suggest that neurogranin processing in  
374 AD may reflect both synaptic and axonal damage. Since neurogranin was associated with tau and  
375 amyloid pathology, it would be interesting to study whether a similar proteolytic pattern is observed  
376 in CJD, where neurogranin levels are altered in brain and CSF tissue without the presence of AD  
377 pathological hallmarks.

378 In total, this study evaluates for the first time the diagnostic and prognostic value of CSF neurogranin  
379 in CJD in comparison to AD. Additionally, we show a striking correlation between brain and CSF  
380 findings regarding different diseases (CJD vs AD) and CJD subtypes (MM1/MV1 vs VV2). This  
381 strongly supports the usefulness of comparative analysis between brain and biological fluids to  
382 comprehensively understand the molecular mechanisms underlying neurodegenerative dementias and  
383 the associate value of their study as diagnostic and prognostic markers for these conditions.

384

#### 385 **Author contributorship:**

386 IZ, IF and FL designed the study. KB, DD-L, HZ, IF, and FL performed experiments. KB, DD-L, HZ,  
387 AV-P, AK, MS, IF and FL analyzed data and interpreted the results. EV provided reagents and  
388 technical expertise. FL wrote the manuscript draft. All authors critically revised the manuscript and  
389 approved its content before submission.

390

#### 391 **Competing interest and funding:**

392 KB has served as a consultant or at advisory boards for Alzheon, CogRx, Biogen, Novartis, and  
393 Roche Diagnostics, unrelated to this work. HZ has served at scientific advisory boards for Eli Lilly,  
394 Roche Diagnostics, Samumed, CogRx and Wave and has received travel support from Teva. KB and  
395 HZ are co-founders of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform  
396 company at the University of Gothenburg. The other authors report no conflicts of interest related to  
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406 2020) programme.

407

408 **Ethical approval:**

409 The study was conducted according to the revised Declaration of Helsinki and Good Clinical Practice  
410 guidelines, and approved by local Ethics committees (Reference numbers 11/11/93, 9/06/08,  
411 Universitaetsmedizin Göttingen, Germany).

412 **Data sharing:**

413 All data relevant to the study are included in the article or uploaded as supplementary information.

414

415

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553 reduced in brain tissue from patients with familial and sporadic Alzheimer's disease. *Acta*  
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555

## 556 **FIGURE LEGENDS**

### 557 **Figure 1. Analysis of CSF neurogranin levels in the differential diagnosis of AD and CJD.**

558 (A) Demographic and biomarker characteristics of the CSF cases used in the present study. Number  
559 of cases, sex (f: female, m: male), age, semi-quantitative analysis of 14-3-3 protein (pos: positive, neg:  
560 negative) and quantitative analysis of neurogranin, total tau (tau) and neurofilament light (NFL)  
561 (mean  $\pm$  standard deviation (SD)) are indicated. NC: Neurological controls, AD: Alzheimer's disease  
562 and CJD: sporadic Creutzfeldt-Jakob disease. (B) Neurogranin concentrations in NC, AD, and CJD.  
563 Neurogranin was significantly different in ND vs AD ( $p < 0.01$ ), NC vs CJD ( $p < 0.001$ ) and AD vs CJD  
564 ( $p < 0.001$ ) comparisons. Statistical significance derived from a multi-comparison analysis for tau,  
565 NFL and neurogranin among the diagnostic groups is indicated. Kruskal-Wallis test followed by  
566 Dunn's post-test (correction for multiple testing) was applied. (C) Diagnostic accuracy of CSF  
567 neurogranin in the discrimination of NC, AD and CJD groups. Area Under the Curve (AUC) with  
568 Standard Error (Strtd. Error) and 95% Coefficient of Interval (CI) derived from Receiver Operating  
569 Characteristic curves for the comparisons between pairs of diagnostic groups is shown. \* $p < 0.05$ ,  
570 \*\* $p < 0.01$  and \*\*\* $p < 0.001$ .

### 571 **Figure 2. Association between neurogranin, demographic and genetic factors in the study** 572 **population in CJD.**

573 (A) No correlation was found between neurogranin levels and age at disease onset in CJD cases. (B)  
574 Neurogranin concentrations did not correlate with sex distribution in CJD cases. Spearman rank  
575 correlation and unpaired t-test analysis were used respectively. (C) Neurogranin concentrations in  
576 CJD stratified by prion protein gene (*PRNP*) codon 129 polymorphism (M = Methionine, V = Valine,  
577 MM,  $n = 38$ , MV:  $n = 13$ , VV:  $n = 14$ ). Kruskal-Wallis test followed by Dunn's post-test (correction for  
578 multiple testing) was applied (\* $p < 0.05$  for MM vs VV and MV vs VV comparisons). (D) Neurogranin  
579 concentrations in sCJD MM1/MV1 ( $n = 15$ ) and VV2 ( $n = 9$ ) subtypes. Unpaired t-test analysis was  
580 applied (\*\* $p < 0.01$  for MM1/VV1 vs VV2 comparison).

### 581 **Figure 3. Association between neurogranin, prion biomarkers and clinical data in CJD.**

582 (A) Correlation analysis between neurogranin, tau and NFL concentrations in CJD cases. Spearman's  
583 rho and p values are indicated for each comparison. Positive significant associations were detected  
584 between neurogranin and tau ( $p < 0.001$ ) and between tau and NFL ( $p < 0.01$ ). (B) Neurogranin  
585 concentrations in CJD stratified by 14-3-3 protein testing outcomes. Negative test was considered  
586 when absence or trace of 14-3-3 protein was detected in the western blot analysis. Mann-Whitney U  
587 test was used. CJD cases with positive 14-3-3 test displayed higher neurogranin concentrations than  
588 CJD cases with negative 14-3-3 test (\* $p < 0.05$ ). (C) Neurogranin concentrations stratified by disease  
589 stage (early, middle and late) in CJD cases. No statistical differences between disease stages were



590 detected. Kruskal-Wallis test followed by Dunn's post-test (correction for multiple testing) was  
591 applied. (E) Association between neurogranin concentrations and disease duration (months) in CJD  
592 patients using a fractional polynomial approach based on a linear regression model. Disease duration  
593 can be modelled as a function of neurogranin values based on the formula: neurogranin (in g/ml) =  
594  $533 + 1/(47*[\text{survival time in months}-1.6]) - 28*[\text{survival time in months}-0.6]$ .

595 **Figure 4. Neurogranin expression in control brain tissue.**

596 (A) Immunohistochemical analysis of neurogranin expression in the cerebral cortex (n=13), white  
597 matter (n=13), cerebellum (n=8) and hippocampus (n=6) of control brain tissue. Neurogranin  
598 immunoreactivity was present in the cerebral cortex and hippocampus and absent in white matter and  
599 cerebellum regions. Bar: 50  $\mu$ m. (B) Cell fractionation analysis of human frontal cortex cases (n=4)  
600 by differential centrifugation. Input and cell fractions (Cyt: cytoplasm, Memb: membrane, PSD: post-  
601 synaptic-density) were separated by SDS-PAGE, followed by immunoblotting with neurogranin,  
602 PSD-95, ATPase Na/K $\beta$ , GAPDH and synaptophysin antibodies as specific markers of each cellular  
603 fraction (left panel). Quantification analysis relative to the % of protein detected in each cell fraction  
604 is indicated (right panel).

605 **Figure 5. Neurogranin expression in AD and CJD brain tissue.**

606 (A) Immunohistochemical analysis of neurogranin expression in the cerebral cortex and hippocampus  
607 of control, CJD and AD brain tissue. Bar: 50  $\mu$ m. (B) Quantification of immunohistochemical staining  
608 of neuronal neurogranin from figure 5A. Cerebral cortex: control; n=10, AD; n=10, CJD; n=9.  
609 Hippocampus: control; n=6, AD; n=7, CJD; n=5. Neurogranin expression in both regions was  
610 decreased in controls compared to AD and CJD ( $p < 0.001$  for all the comparisons) and in AD  
611 compared to CJD ( $p < 0.01$  in cerebral cortex and  $p < 0.05$  in hippocampus). ANOVA test followed by  
612 Tukey's post-hoc was applied. \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ . (C) Quantification of  
613 immunohistochemical analysis from AD cases according to Braak stage. AD IV; n=3, AD V; n=4;  
614 AD VI; n=3. ANOVA test followed by Tukey's post-hoc was applied.

615 **Figure 6. Neurogranin expression in CJD and association with synaptic and axonal markers. (A)**

616 Western blot analysis of PSD-95, tau, synaptophysin, neurogranin and  $\beta$ -actin in the frontal cortex of  
617 control, sCJD MM1 and sCJD VV2 cases. A representative image (4 controls, 5 CJD MM1 and 5  
618 CJD VV2) is shown. (B) Quantification of the western blot analysis from the complete cohort of cases  
619 analyzed, which included: controls; n=8, CJD MM1; n=10 and CJD VV2; n=10. ANOVA test  
620 followed by Tukey's post-hoc was applied. PSD-95, tau, synaptophysin and neurogranin levels was  
621 reduced in CJD cases compared to controls (\* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ ). (C) Correlation  
622 analysis of Neurogranin with tau, synaptophysin and PSD-95 in CJD cases (n=20) (left panel) and  
623 correlation values (rho, 95% CI and p value) for each comparison between pair of proteins (right  
624 panel).

625 **Figure 7. Neurogranin levels in AD and association with synaptic and axonal markers.**

626 Western blot analysis of PSD-95, tau, synaptophysin, neurogranin and  $\beta$ -actin in the frontal cortex of  
627 control, and AD cases. A representative image (4 controls and 4 AD) is shown. (B) Quantification of  
628 the western blot analysis from the complete cohort of cases analyzed (controls; n=23, AD; n=18).  
629 ANOVA test followed by Tukey's post-hoc was applied. PSD-95/synaptophysin and neurogranin  
630 expression was reduced in AD cases compared to controls (\* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ ). (C)  
631 Correlation analysis of Neurogranin with tau, synaptophysin and PSD-95 in AD cases (n=18) (left  
632 panel) and correlation values (rho, 95% CI and p value) for each comparison between pair of proteins  
633 (right panel).

634

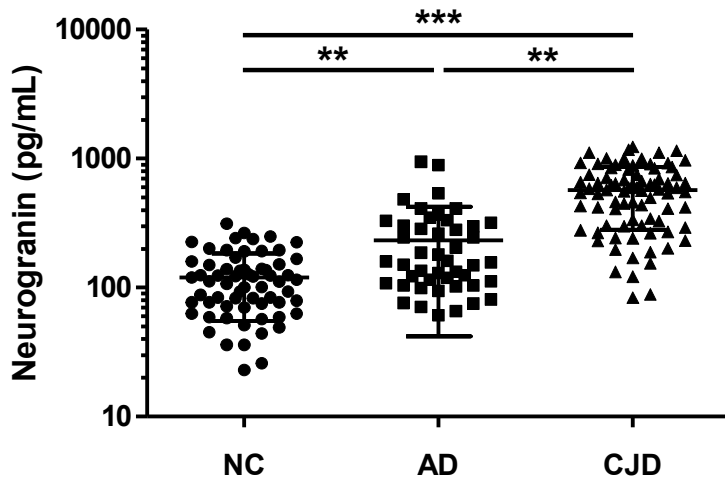
635 **Supplementary Table 1. Demographic, neuropathological genetic characteristics of the brain**  
636 **cases used in the present study.** (A) Controls, (B) AD and (C) CJD. Number of cases, age at onset,  
637 sex (f: female, m: male), and post-mortem time delay (PMT) is indicated. Braak neurofibrillary tangle  
638 (NFT) stage in AD cases and CJD subtype in CJD cases is indicated. FC(R8): frontal cortex  
639 Brodmann region 8, HPC: hippocampus, CB: cerebellum. IHC: Immunohistochemistry, WB: Western  
640 blot, PSD: Post-synaptic density. 0 and B refers to amyloid stage.

**Figure 1**

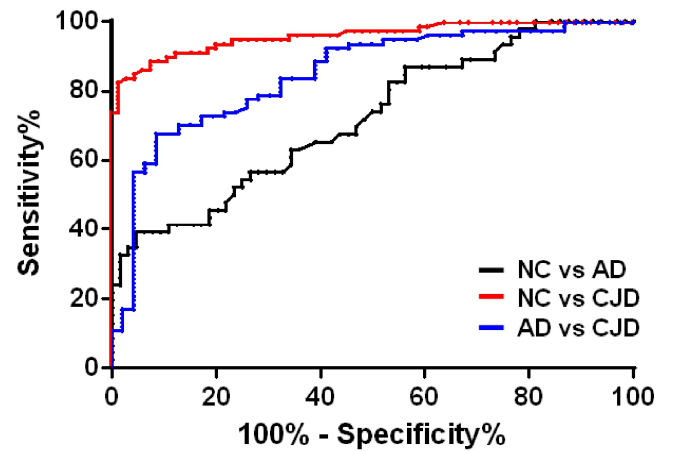
**A**

	<i>NC</i>	<i>AD</i>	<i>CJD</i>
<i>Number of cases</i>	64	46	81
<i>Sex (number, f/m)</i>	33/31	27/19	47/34
<i>Age (mean ± SD, years)</i>	65 ± 11	66 ± 9	67 ± 10
<i>14-3-3 (number, pos/trace/neg)</i>	3/4/57	9/3/34	72/4/5
<i>Neurogranin (mean ± SD, pg/mL)</i>	120 ± 65	233 ± 191	571 ± 291
<i>tau (mean ± SD, pg/mL)</i>	243 ± 204	747 ± 606	10017 ± 8541
<i>NFL (mean ± SD, pg/mL)</i>	1391 ± 2112	1851 ± 1537	5919 ± 4229

**B**

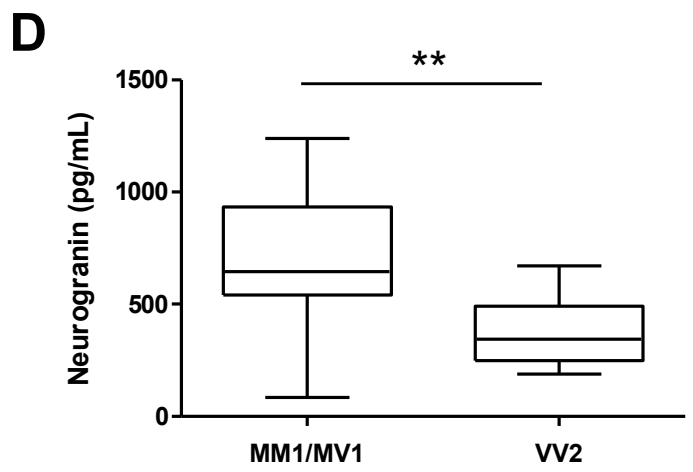
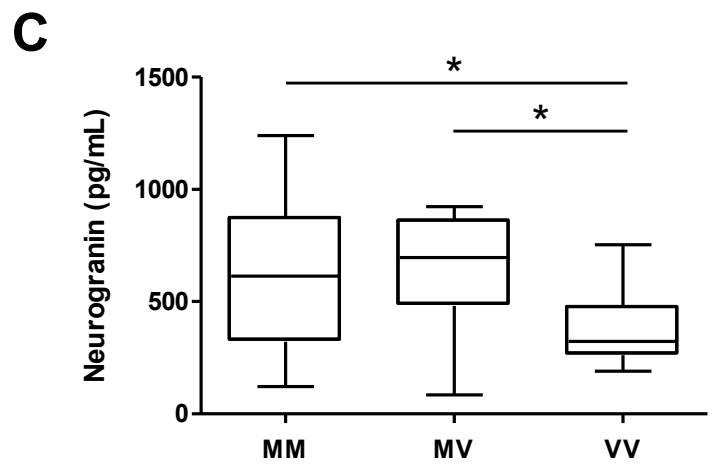
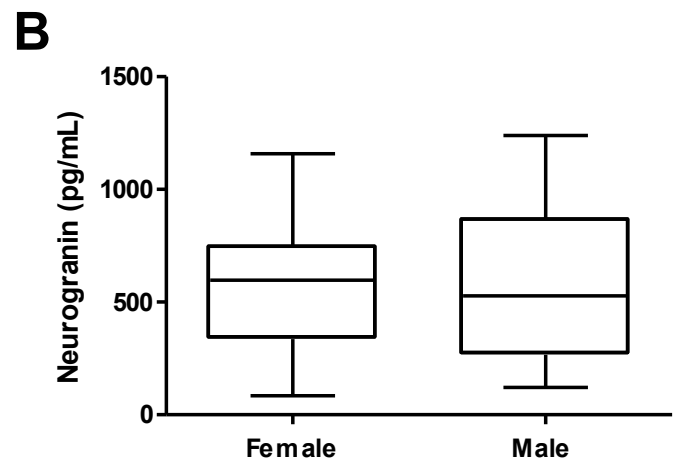
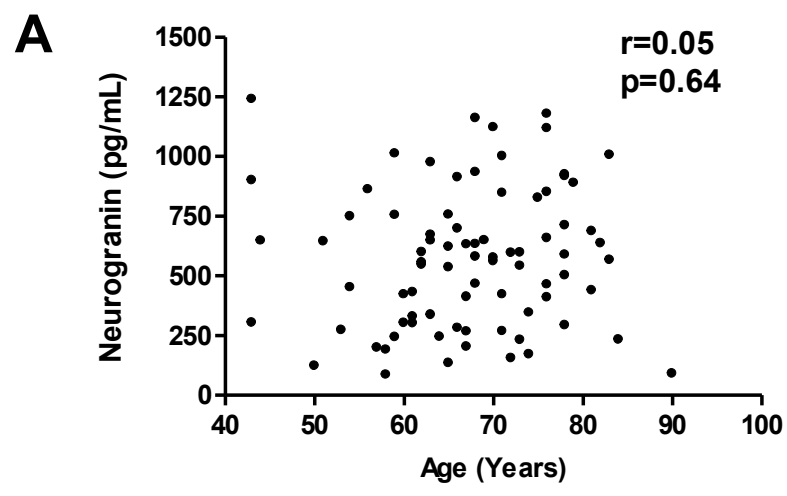


**C**

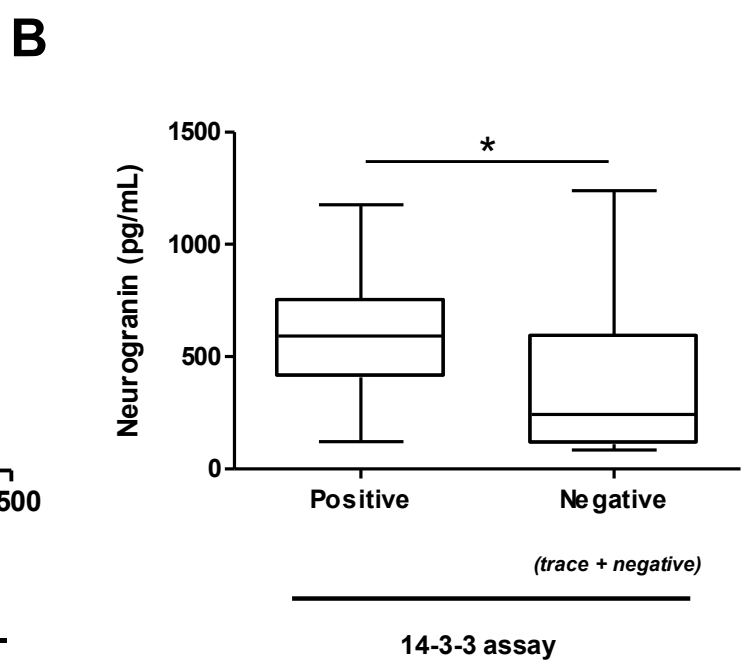
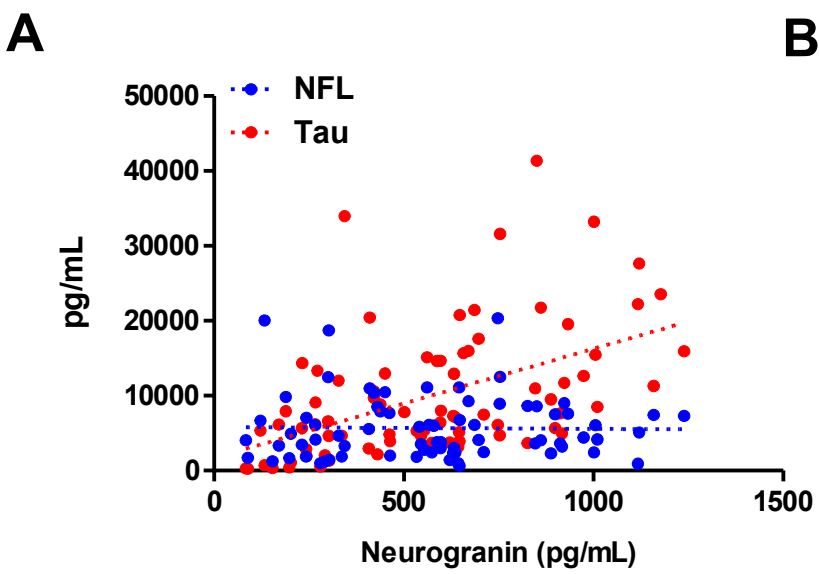


	<i>NC vs AD</i>	<i>NC vs CJD</i>	<i>AD vs CJD</i>
Area	0.73	0.96	0.85
Std. Error	0.05	0.01	0.03
95% CI	0.62-0.82	0.93-0.99	0.78-0.92

Figure 2



**Figure 3**



	<i>rho</i>	95% CI	<i>p</i> value
Neurogranin vs Tau	0.55	0.37-0.69	< 0.0001
Neurogranin vs NFL	0.09	-0.15-0.31	0.46
Tau vs NFL	0.28	0.05-0.49	0.01

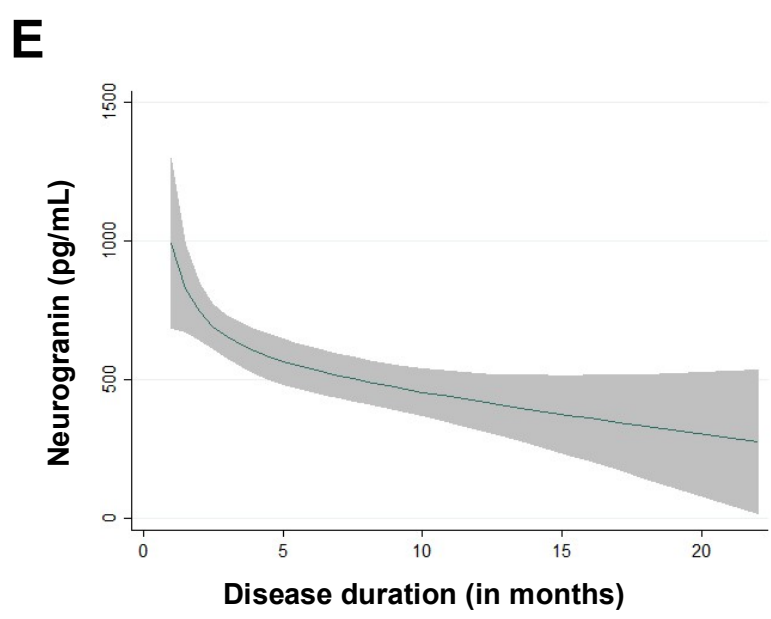
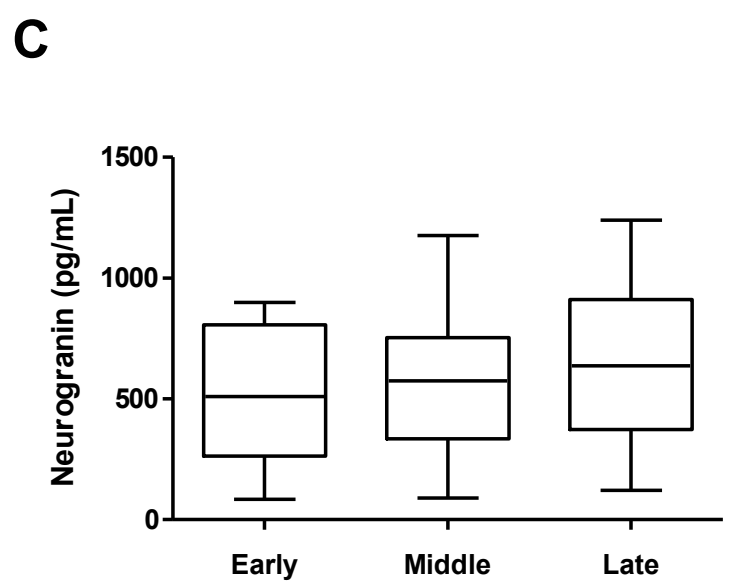


Figure 4

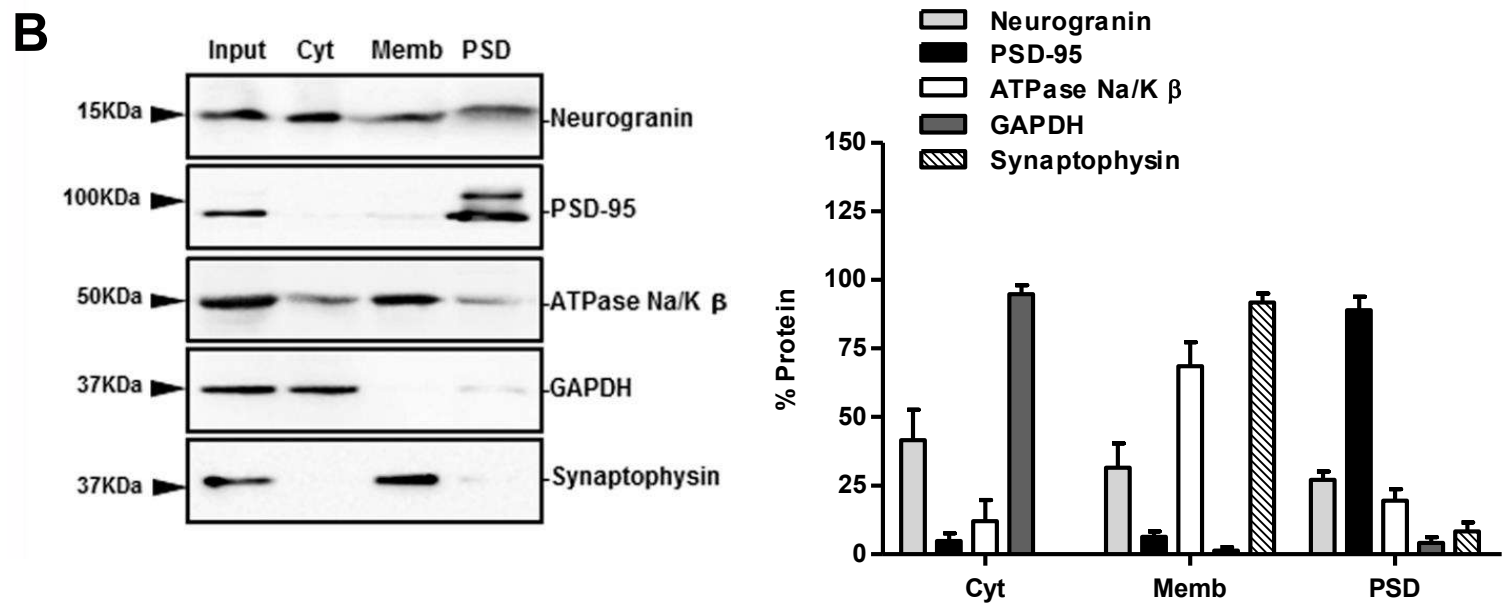
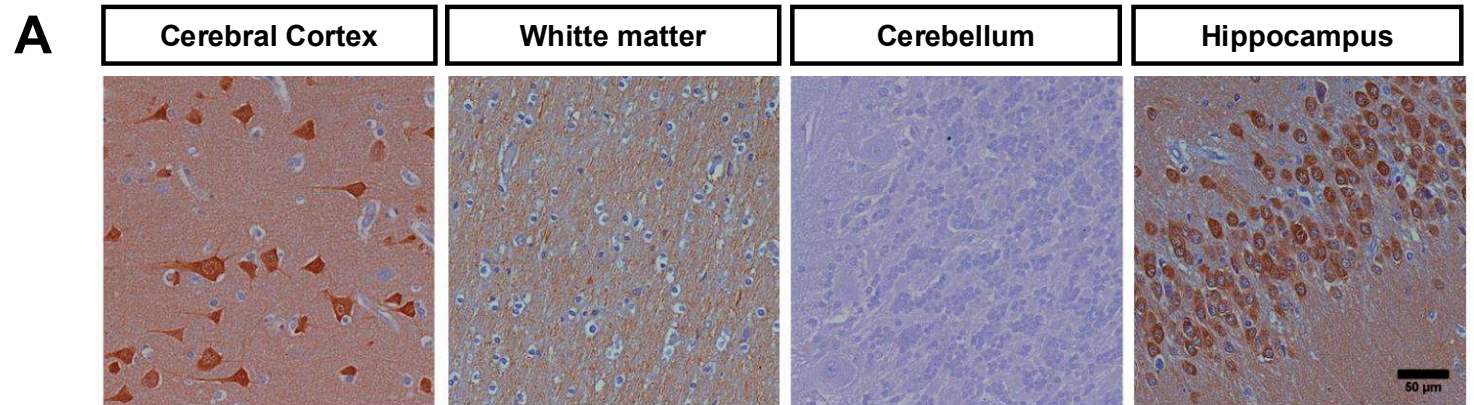
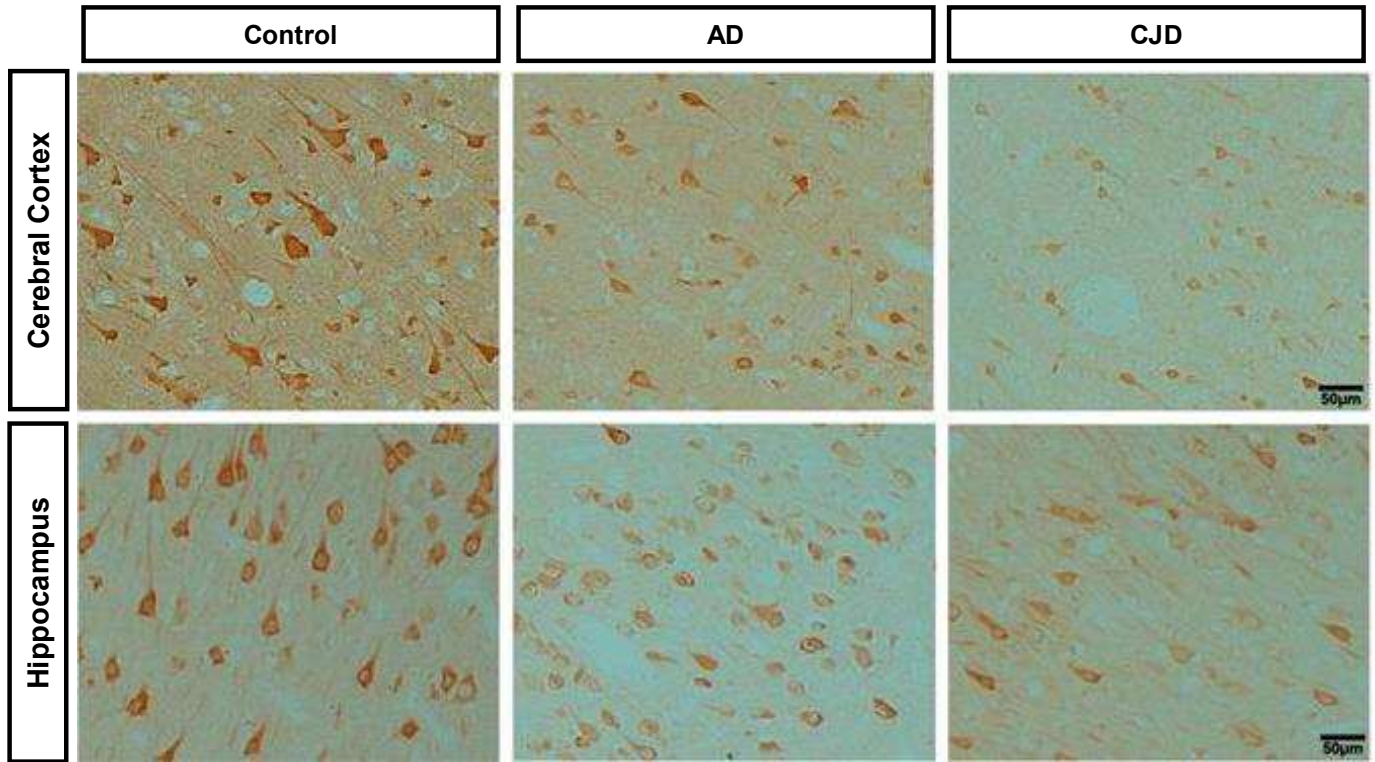
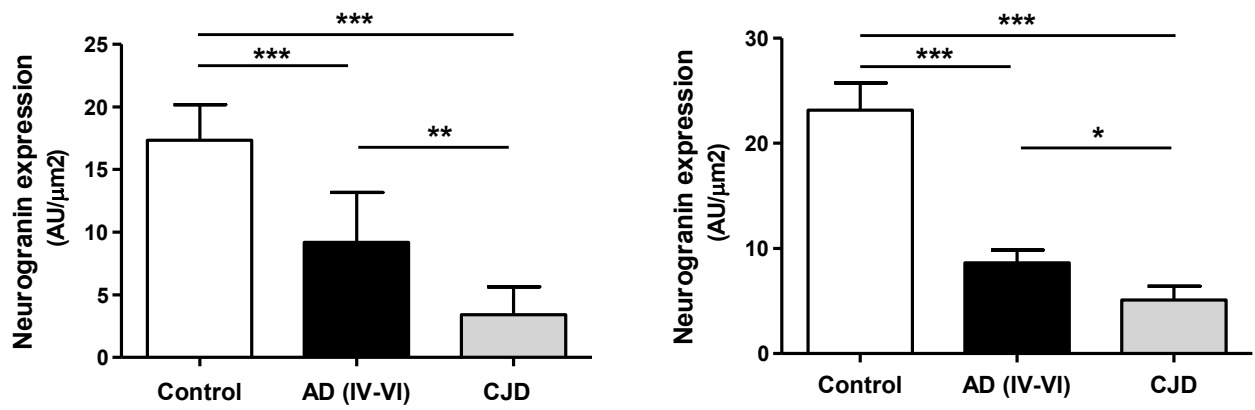


Figure 5

A



B



C

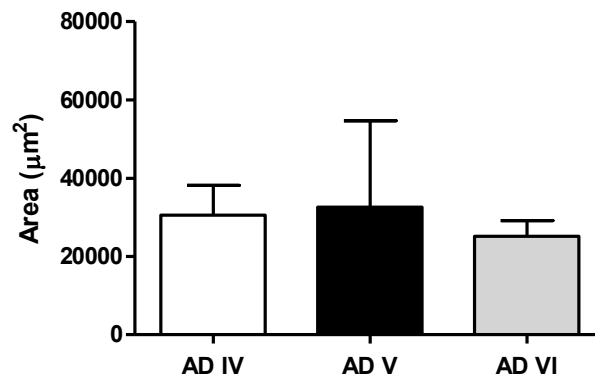
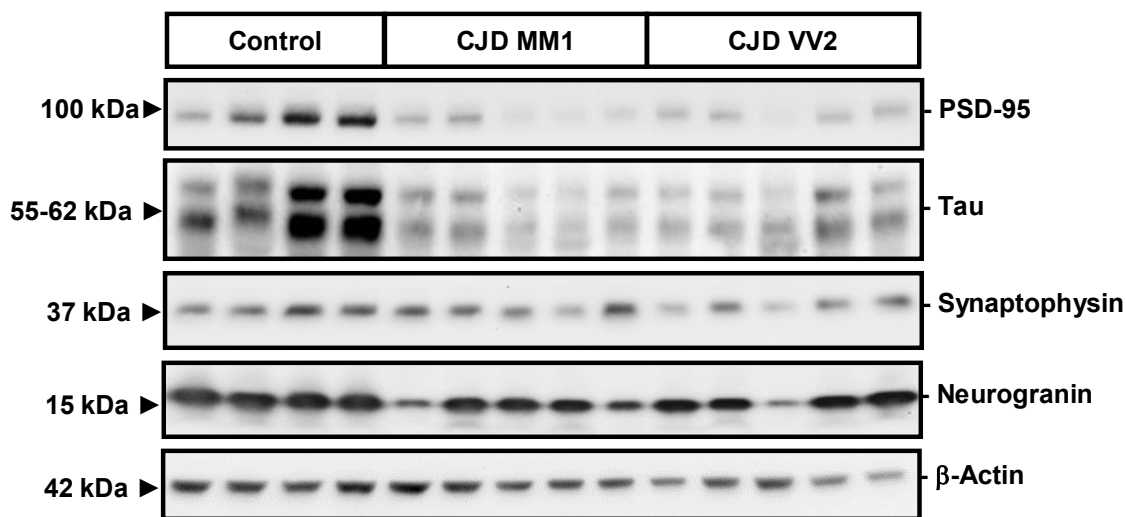
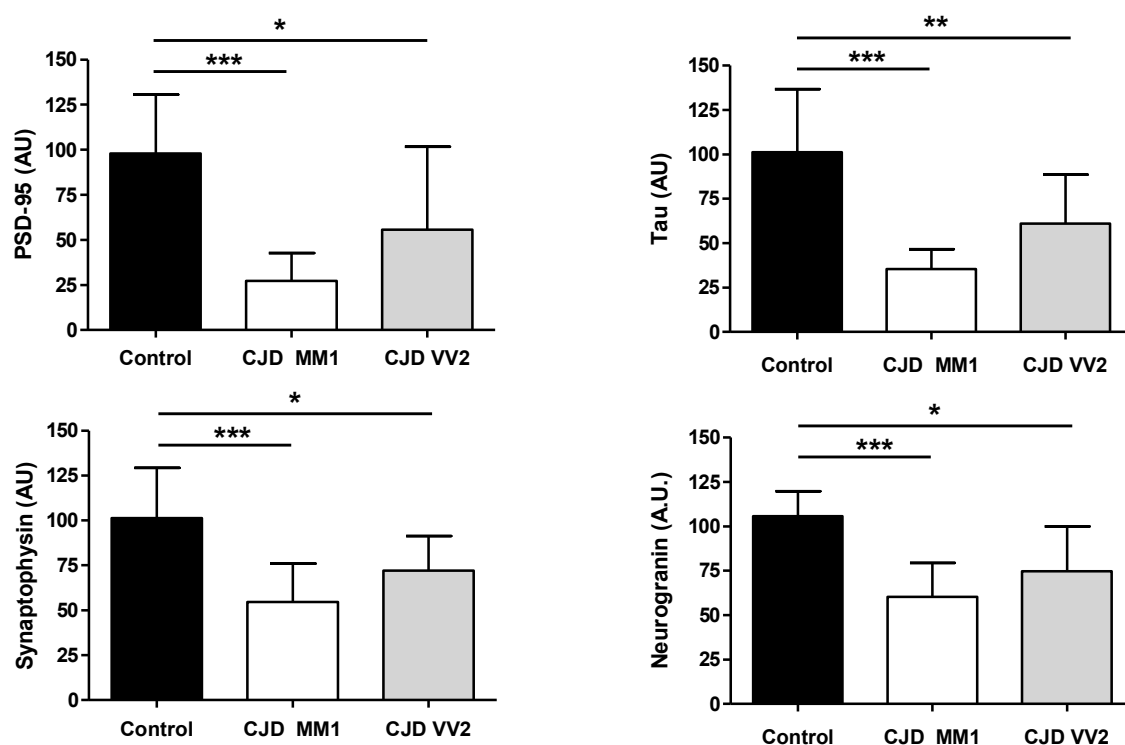


Figure 6

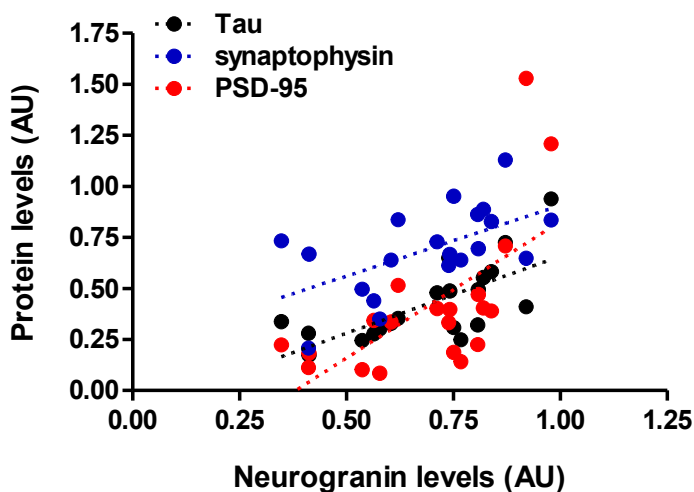
A



B



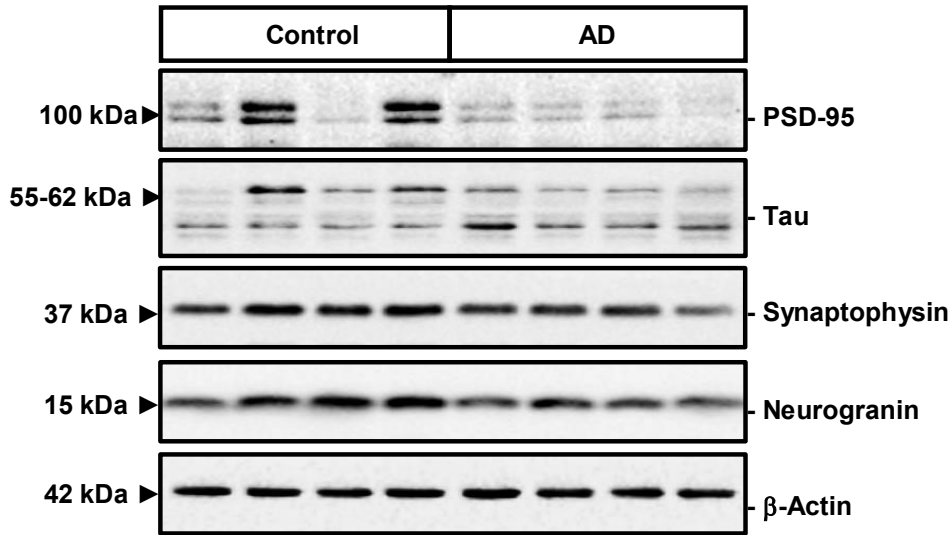
C



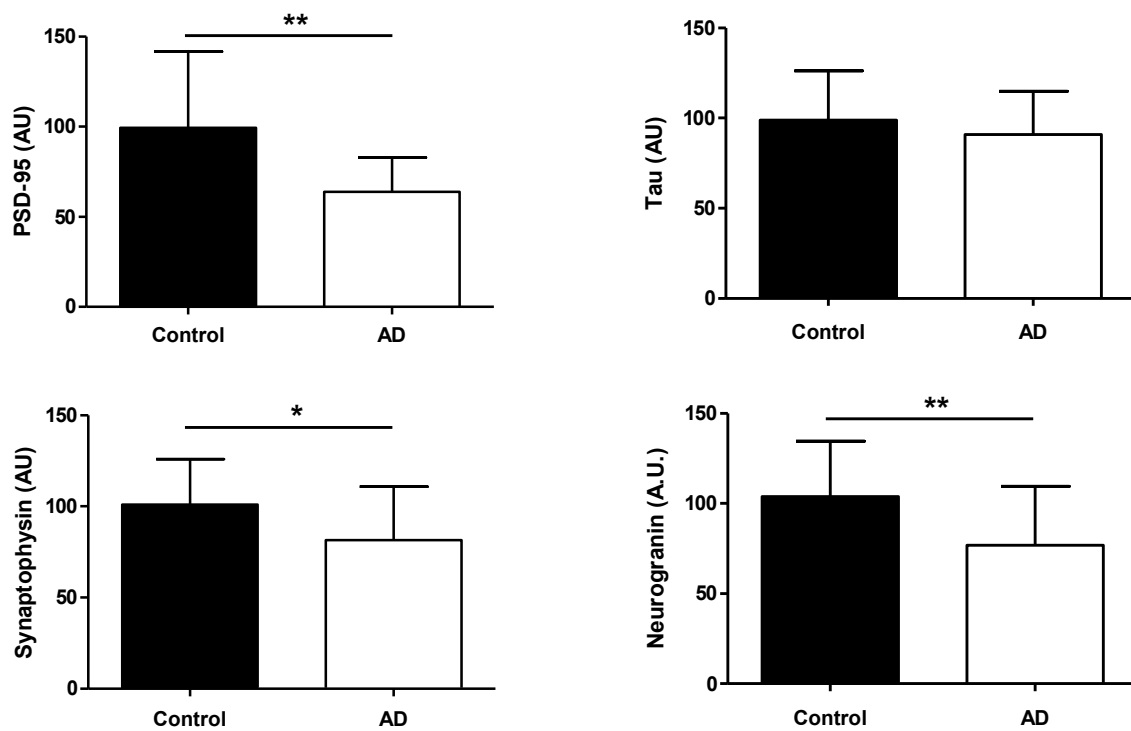
	<i>rho</i>	95% CI	<i>p</i> value
Neurogranin vs Tau	0.69	0.34-0.87	< 0.001
Neurogranin vs synaptophysin	0.55	0.14-0.80	0.01
Neurogranin vs PSD-95	0.69	0.35-0.87	< 0.001
Tau vs synaptophysin	0.52	0.09-0.79	0.02
Tau vs PSD-95	0.76	0.47-0.90	< 0.001
PSD-95 vs synaptophysin	0.53	0.10-0.79	0.02



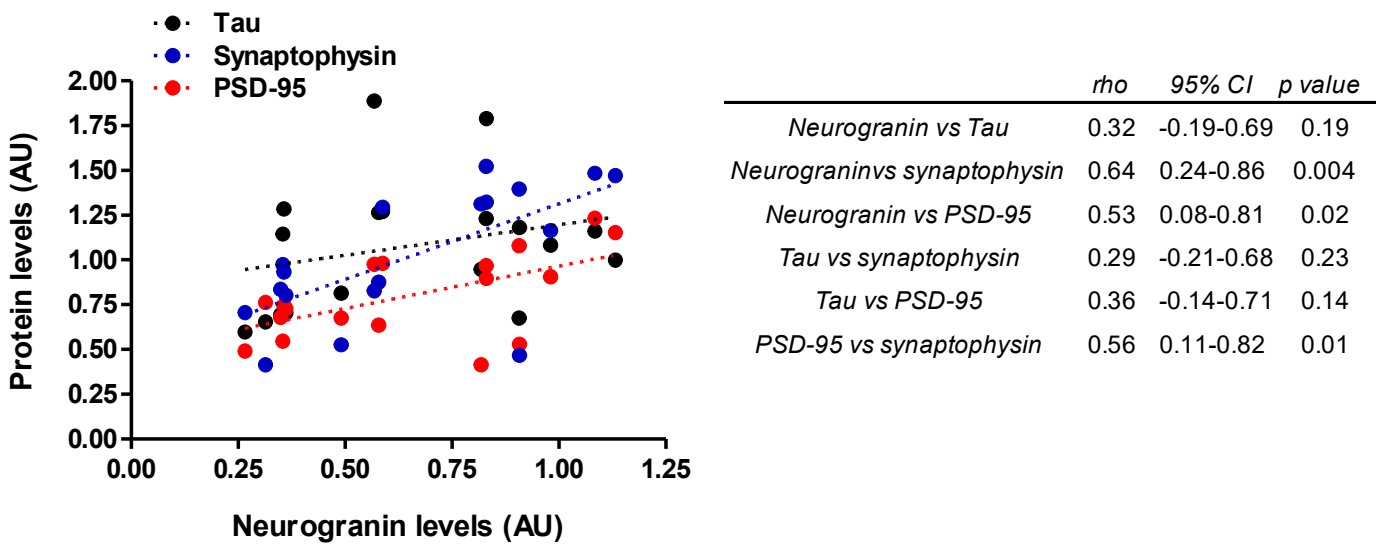
A



B



C



A

## Supplementary Table 1

Frontal Cortex	ID	Age (years)	Sex	PMT (hours)	Neuropathological findings	IHC	WB	PSD
	1	41	F	12h	Not available		X	
	2	53	M	3h	No neuropathological lesions	X	X	
	3	70	M	12h	No neuropathological lesions	X	X	
	4	55	M	5h 40m	Not available		X	
	5	59	M	7h 5m	No neuropathological lesions			X
	6	39	M	9h 15m	Infarction, lacunar	X		X
	7	46	F	14h 5m	Not available		X	
	8	59	M	6h 25m	No neuropathological lesions		X	
	9	71	F	8h 30m	Neurofibrillary tangle pathology I, 0		X	
	10	43	M	5h 55m	No neuropathological lesions		X	
	11	39	M	3h 30m	Argyrophilic grain disease III	X	X	X
	12	56	M	3h 50m	Multi-infarct		X	
	13	71	M	15h	Neurofibrillary tangle pathology I, 0		X	
	14	79	F	3h 35m	Neurofibrillary tangle pathology I, 0			X
	15	55	M	2h 45 m	Infarction, lacunar	X		
	16	49	M	09h 25 m	Neurofibrillary tangle pathology, scant	X		
	17	62	M	19h 55 m	No neuropathological lesions	X		
	18	50	F	14h 30 m	Hypoxia, hippocampus	X		
	19	72	F	8h 30m	Neurofibrillary tangle pathology II, 0		X	
	20	50	M	17h 15m	No neuropathological lesions	X		
	21	54	F	6h 45m	Ischaemic changes hippocampus			
	22	52	M	4h 40m	No neuropathological lesions	X	X	
	23	52	F	5h 45m	No neuropathological lesions		X	
	24	61	M	4h 30m	Neurofibrillary tangle pathology I, 0		X	
	25	45	M	4h 5m	Cerebral infarction		X	
	26	77	M	6h 55m	No neuropathological lesions		X	
	27	73	M	9h 35m	Neurofibrillary tangle pathology II, 0		X	
	28	65	F	15h	Neurofibrillary tangle pathology II, B		X	
	29	60	F	11h 30m	No neuropathological lesions		X	
	30	72	M	15h 55m	Argyrophilic grain disease III		X	
	31	63	M	4 h 5 min	Hypoxia		X	
	32	41	M	11h 35m	Small vessel disease	X	X	
	33	78	M	12h	Small vessel disease		X	
	34	72	F	4h	Status cribosus		X	
	35	59	M	21h 35m	No neuropathological lesions	X	X	
	36	59	M	8h 30m	Status cribosus	X	X	
	37	54	M	8h 45m	No neuropathological lesions		X	
	38	72	M	4h 20m	No neuropathological lesions		X	
	39	70	M	13h	No neuropathological lesions		X	
	40	63	M	17h	No neuropathological lesions		X	
	41	59	M	7h	No neuropathological lesions		X	

Hippocampus	ID	Age (years)	Sex	PMT (hours)	Neuropathological findings	IHC	WB	PSD
	1	53	M	15h 20m	Neurofibrillary tangle pathology, scant	X		
	2	63	M	17h	No neuropathological lesions	X		
	3	53	M	3h	No neuropathological lesions	X		
	4	55	M	2h 45m	Infarction, lacunar	X		
	5	40	M	5h 10m	Neurofibrillary tangle pathology, scant	X		
	6	54	M	8h 45m	Calcifications	X		

Cerebellum	ID	Age (years)	Sex	PMT (hours)	Neuropathological findings	IHC	WB	PSD
	1	39	M	9h 15m	No neuropathological lesions	X		
	2	23	M	12h 55m	No neuropathological lesions	X		
	3	52	M	04h 40 m	No neuropathological lesions	X		
	4	52	F	05h 45 m	No neuropathological lesions	X		
	5	62	M	3h 30m	Hematoma	X		
	6	42	M	4h 20m	No neuropathological lesions	X		
	7	54	M	8h 45m	Calcifications	X		
	8	40	M	18h 30m	No neuropathological lesions	X		

Frontal Cortex	ID	Age (years)	Sex	PMT (hours)	NFT (Braak Stage)	IHC	WB
	1	75	M	6h 10m	IV		X
	2	79	M	5h	IV	X	
	3	79	M	4h 15m	IV		X
	4	89	M	3h 20m	IV	X	X
	5	84	M	26h	IV	X	
	6	72	F	9h 30m	V		X
	7	86	M	4h 15m	V		X
	8	82	F	1h 45m	V		X
	9	73	M	4h 30m	V	X	
	10	85	F	16h 15m	V	X	
	11	93	M	3h	V		X
	12	75	M	11h 30m	V		X
	13	81	F	5h 15m	V		X
	14	77	M	NA	V	X	
	15	74	F	9h	V		X
	16	50	M	9h 15m	V	X	
	17	69	M	13h 10m	V-VI		X
	18	64	M	6h 10m	V-VI		X
	19	67	F	6h 10m	V-VI		X
	20	82	M	5h	V-VI		X
	21	86	F	20 h 35 m	VI	X	X
	22	67	F	8h	VI		X
	23	56	F	7 h	VI	X	X
	24	88	M	4h 45m	VI	X	X
Hippocampus	ID	Age (years)	Sex	PMT (hours)	NFT (Braak Stage)	IHC	WB
	1	72	F	16h 10m	IV	X	
	2	84	M	26h	IV	X	
	3	75	M	11h 30m	V	X	
	4	88	M	4h 45m	V	X	
	5	50	M	9h 15m	V	X	
	6	84	F	21h	V	X	
	7	56	F	7h	VI	X	

Frontal Cortex	ID	Age (years)	Sex	PMT (hours)	CJD Subtype	IHC	WB
	1	44	M	6h 30 m	MM1	X	
	2	46	M	4h 45m	MM1	X	X
	3	59	M	21 h	MM1	X	X
	4	57	M	4h 15 m	Not available	X	
	5	56	F	13h 30m	MM2	X	
	6	61	M	30h 30m	MM1	X	
	7	64	M	40h	Not available	X	
	8	66	M	NA	VV2	X	
	9	59	M	10h	MM2	X	
	10	72	F	8h	MM1		X
	11	85	F	NA	MM1		X
	12	59	F	15h	MM1		X
	13	60	F	5h 30m	MM1		X
	14	65	F	5h 30m	MM1		X
	15	76	M	18h 30m	MM1		X
	16	78	M	23h	MM1		X
	17	25	M	4h	MM1		X
	18	66	M	5h	VV2		X
	19	71	M	9h	VV2		X
	20	76	F	5h	VV2		X
	21	76	F	5h 30m	VV2		X
	22	51	F	6h	VV2		X
	23	65	M		VV2		X
	24	73	F	24h	VV2		X
	25	47	F	5h 30m	VV2		X
	26	54	M	9h	VV2		X
	27	65	F	7h	VV2		X
Hippocampus	ID	Age (years)	Sex	PMT (hours)	CJD Subtype	IHC	WB
	1	57	M	4 h 15 m	MM1	X	
	2	56	F	13h 30m	MM2	X	
	3	72	F	17h	MM1	X	
	4	74	M	2h	MM1	X	
	5	61	M	30h 30m	Not available	X	