Cerebrospinal fluid neurogranin concentrations in Alzheimer's disease and major depressive disorder

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Abstract

Objective: Synaptic dysfunction is linked to both Alzheimer's disease (AD) and major depressive disorder (MDD). Synapse protein concentrations in cerebrospinal fluid (CSF) may be useful biomarkers to monitor synaptic dysfunction and degeneration that lead to depressive symptoms and AD, respectively. CSF neurogranin (Ng), a post-synaptic protein, has emerged as a promising tool to detect synaptic dysfunction and/or loss in AD. The aim of this study was to test the specific hypothesis that CSF neurogranin (Ng) is able to differentiate AD from MDD and cognitively normal controls.

Method: CSF samples from 44 healthy control individuals (CTRL) and 86 mild cognitive impairment (MCI, 36 of whom had prodromal AD, as defined by a positive CSF AD biomarker signature), 25 AD and 6 MDD subjects were analyzed using an in house enzyme-linked immunosorbent assay (ELISA) for Ng.

Results: CSF Ng levels were significantly higher in AD patients and in prodromal AD (MCI patients with an "AD-like" CSF tau and Aβ42 profile) compared with CTRL individuals ($p<0.0001$ for both groups) and MDD patients ($p<0.001$ and $p<0.01$, respectively). Significantly higher CSF Ng concentration was also seen in prodromal AD patients as compared to MCI patients without biomarker evidence of underlying AD pathology ($p<0.0001$). CSF Ng correlated positively with the classical axonal injury markers CSF T-tau and P-tau ($p<0,0001$), whereas correlation to plaque pathology as reflected by CSF Aβ42 was less clear. Negative correlations of CSF Ng with cognitive evaluation scores (MMSE and CAMCOG) were observed.

Conclusion: This study strengthens the clinical utility of CSF Ng as a CSF biomarker for AD. AD patients in both MCI and dementia stages of the disease had increased CSF Ng concentrations compared with cognitively normal control individuals, patients with non-AD MCI and patients with MDD. The lowest CSF Ng concentrations were seen in patients with MDD, a finding that warrants validation in further studies.

Key words: Cerebrospinal fluid, neurogranin, Alzheimer's disease, major depressive disorder, biomarkers.

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder. It is characterized by pathological hallmarks including neuronal and synaptic degeneration and loss together with deposits of aggregated amyloid-β (Aβ) and tau[1]. Lower cerebrospinal fluid (CSF) concentrations of the 42 amino acid–long Aβ peptide Aβ$_{1-42}$, reflecting the plaque pathology, together with increased concentrations of total tau (T-tau) and phosphorylated tau (P-tau), reflecting neurodegeneration and tangle pathology, respectively, are today considered the core CSF biomarkers for AD[1]. Synaptic loss has been identified as an early event in the disease progression, as well as the underlying cause of the progressive cognitive deterioration as the disease advances[2], and recently CSF neurogranin (Ng), a post-synaptic protein that is enriched especially in dendritic

spines, has emerged as a promising tool to detect synaptic dysfunction and/or loss in AD. CSF Ng concentrations are increased in AD, already in the pre-dementia (mild cognitive impairment, MCI) stage of the disease, and correlate with hippocampal atrophy and cognitive decline over time[3-10]. Across neurodegenerative diseases CSF Ng increase seems to be specific to AD[11]

Here, we aimed at replicating the association of CSF Ng with AD in a mono-centre cohort of well-characterised AD patients in different stages of the disease. We also report, for the first time, CSF Ng concentrations in patients with major depressive disorder (MDD), a common differential diagnosis to AD, which also may involve synaptic dysfunction[12]

2. Materials and Method

2.1 Study population

CSF samples from patients recruited in the Department of Psychiatry, University of Sao Paulo, Sao Paulo Brazil were collected during a period of 4 years. Patients diagnosed with AD fulfilled the dementia criteria of the Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revision, and the criteria for probable AD as defined by NINCDS-ADRDA[13]. We re-selected a cohort of the patients where we included 44 healthy control (CTRL), 86 mild cognitive impairment (MCI), 25 AD, 6 MDD subjects (see Table 1 for full biomarker and demographic characteristics) discriminated by using a cut off established by a ROC curve towards $A\beta_{1-42}$ (<390 pg/ml with a Se=64% and Spe=68% with a Youden index=0.97[14]) and T-tau level (>84 pg/ml with a Se=68% and Spe=68% with a Youden Index=1[14]). Applying this cut-off in the MCI group we observed a clear separation in two groups, according CSF biomarkers profile, one closer to the CTRL profile (MCI=50) and the other one closer to the AD profile (prodromal AD=36). All individuals underwent brain imaging, routine laboratory testing and neurological, psychiatric and cognitive examinations. The study was approved by the local ethics committee (University of Sao Paulo). All subjects gave written informed consent. The study was conducted according to the provisions of Declaration of Helsinki. For details on the number of cases in each diagnostic group, the demographic and clinical characteristics see Table 1A.

2.2 Cognitive assessments

Global cognition was assessed by Mini Mental State Examination (MMSE)[15] and Cambridge Cognition Examination (CAMCOG)[16].

2.3 Cerebrospinal fluid collection and biomarker analysis

Patients consented to undergo lumbar puncture for CSF sampling and biomarker analysis. CSF samples were taken by lumbar puncture in the L3/L4 or L4/L5 intervertebral space, with a 23-gauge needle and using polypropylene tubes, in the morning. A total of 12–15 ml of CSF was collected and, then, centrifuged at 3200g for 10min at 4 °C. After centrifugation, the samples were separated in 0.5ml aliquots, and immediately frozen at −80 °C until analysis.
The determination of CSF concentrations of AD-related biomarkers (T-tau, P-tau and $A\beta_{1-42}$), was done in duplicate with the INNo-Bia AlzBio3 assay (Innogenetics, Ghent, Belgium), a multiplex microsphere-based Luminex (xMAP) platform that allows the simultaneous analysis of these biomarkers. These analyses were performed at the University of Sao Paulo. CSF Ng measurement was performed by board-certified laboratory technicians who were blinded to clinical information using an in house ELISA, as previously described in detail[3].

3. Statistical analysis

For statistical analysis, Prism 7 for Mac software (GraphPad Software, La Jolla, CA, USA) and SPSS 20.0

for Mac were used. Based on Shapiro-Wilk test, almost all data were skewed, so nonparametric tests were used. Differences between groups were assessed using the Mann–Whitney U test, and Kruskal-Wallis test was performed to compare data between all groups. Correlations were determined using Spearman's ρ correlation. All tests were two-sided and significance was determined at $P < 0.05$. Association between the levels of CSF Ng, the other CSF biomarkers and the diagnostic groups were tested with linear regression.

4. Results

4.1 CSF Ng concentrations in different diagnostic groups

CSF Ng concentrations were significantly higher in AD patients and in patients with prodromal AD (MCI patients with an "AD-like" CSF profile) compared to CTRL ($p<0.0001$ for both groups) and to MDD ($p<0.001$ and $p<0.01$, respectively). There was also a difference in CSF Ng concentration between patients with MCI without biomarker evidence of underlying AD pathology and prodromal AD patients ($p<0.0001$), confirming that increased CSF Ng concentrations are tightly linked to AD pathology (Table 1A).

4.2 CSF Ng concentrations in relation to "core AD biomarkers"

Ng levels showed a strong positive correlation with both T-tau and P-tau ($r_s=0.68$ $p<0.0000001$; $r_s= 0.60$ $p<0.0000001$, respectively). These correlations were also seen when analysing each diagnostic group separately (Fig.1 or Table 2), there was a negative correlation with $A\beta_{1-42}$ ($r_s= -0.47$ $p<0,0000001$; Table 1B) in the whole material, but this potential correlation was not seen in the diagnostic groups, when analysed separately (Fig.1 or Table 2).

4.3 CSF Ng concentrations in relation to clinical characteristics

Weak negative correlations of CSF Ng with MMSE and CAMCOG were seen in the whole material ($r_s= -0.24$ $p<0.003$; $r_s= 0.23$ $p<0.004$, respectively), but these potential correlations were not significant when examined in the diagnostic groups separately (except in AD group $r_s= -0.40$ $p<0.05$). No significant correlation with age ($r_s= -0.02$ $p= 0.81$) was found (Fig. 3; Table 2). In contrast, CSF $A\beta_{1-42}$, T-tau and P-tau showed weak correlations with age ($r_s= -0.22$ $p= 0.009$; $r_s= 0.20$ $p<0.01$, respectively) in the whole material (Table 1B) but not within diagnostic groups when tested separately (Fig. 1 or Table 2).

Discussion

In the present paper, we show that CSF Ng concentrations are elevated in AD, also in pre-dementia stages of the disease. We also corroborate earlier findings that CSF Ng correlates with neurodegeneration and tau pathology, as reflected by CSF T-tau and P-tau concentrations[17]. These markers are, however, also influenced by age, a potential confound that does not seem to be that important for CSF Ng, given the lack of correlation of CSF Ng with age. Finally, we report for the first time on CSF Ng in MDD. Our cohort only included 6 individuals with MDD but all of these had low CSF Ng concentrations. Although the MDD group is small and the results need to be replicated in larger numbers of patients, we find it tempting to speculate that CSF Ng concentrations may depend both on increased release from degenerating synapses in AD and on reduced physiological release from synapses in MDD, which potentially reflects decreased synaptic activity in the latter condition. This reduction was not statistically significant when comparing MDD patients with healthy controls, which may indicate lack of power due to the small number of MDD patients but the reduction in comparison with both AD groups (AD dementia and prodromal AD) was clear. Although the MDD results are in need of replication due to the small sample size, our results suggest that AD pathology specifically induces abnormal release of Ng into the CSF, which is not seen in MDD. The latter finding, although preliminar, underscores the clinical utility of this marker.

Table 1.

A) Summary of the demographic, clinical and biomarker data.

	AD	MCI	Prodromal AD	MDD	CTRL
Gender, m/f, (%male)	6/19 (24)	20/30 (40)	14/22 (39)	3/3 (50)	13/31 (29,5)
Age (yr)	76 (67-85)	71 (68-76)[†]	73 (71-76)	73 (68-75)	71 (67,5-75)*[†]
Education (yr)	8 (4-15)	11 (6-16)[††]	5 (4-11)	10,5 (4-13,2)	11 (5-15,5)[†]
MMSE	23 (16,5-26)	28 (26-29)***[††]	26,5 (24,2-27)**	28,5 (27-29)**[†]	29 (27-29)***[††]
CAMCOG	74 (59-89)	92 (84-96)***[††]	84,5 (80,2-91,5)*	91 (88-94)**	94 (90-97)***[††]
Aβ_{1-42}, (pg/ml)	310 (237-355)	554 (448-640)***[††]	293 (232-348)	549 (399-673)***[††]	529 (481-639)***[††]
T-tau, (pg/ml)	144 (102-237)	65 (50-83)***[††]	150 (120-209)	69 (46-104)**[††]	68 (54-81)***[††]
P-tau, (pg/ml)	71 (54-102)	32,5 (25-44)***[††]	73 (60-94)	43 (23-55)**[††]	35 (26-46)***[††]
Ng7, (pg/ml)	687 (474-956)	182,00 (83-310)*** [††]	481 (326-841)	144 (76-306)** [†]	235,50 (171-358)*** [††]

B) Correlation between demographic, clinical and CSF data.

Rho Spearman	Age	Education	MMSE	CAMCOG	Aβ_{1-42}	T-tau	P-tau	Ng
Age (p)	1	-0,225[§§]	-0,085	-0,137	-0,220[§§]	0,204[§]	0,12	0,021
Education	-0,225[§]	1	0,222[§§]	0,415[§§§]	0,149	-0,256[§]	-0,125	-0,14
MMSE	-0,08	0,22[§§]	1	0,660[§§§]	0,331[§§§]	-0,418[§§§]	-0,265[§§]	-0,244[§]
CAMCOG	-0,137	0,415[§§§]	0,660[§§§]	1	0,381[§§§]	-0,409[§§§]	-0,294[§§§]	-0,233[§]
Aβ_{1-42}	-0,220[§§]	0,149	0,331[§§§]	0,381[§§§]	1	-0,548[§§§]	-0,564[§§§]	-0,477[§§§]
T-tau	0,204[§]	-0,256[§]	-0,418[§§§]	-0,409[§§§]	-0,548[§§§]	1	0,731[§§§]	0,686[§§§]
Ptau	0,12	-0,125	-0,265[§§]	-0,294[§§§]	-0,564[§§§]	0,731[§§§]	1	0,607[§§§]
Ng	-0,021	-0,141	-0,244[§]	-0,233[§]	-0,477[§§§]	0,686[§§§]	0,607[§§§]	1

A) The values presented are median (IQR). *P<0,5 versus AD; **P<0,01 versus AD; ***P <0,001 versus AD; †P <0,05 versus prodromal AD; †† P<0,01 versus prodromal AD. B) §P<0.01; §§p<0,001; §§§p<0.0001.
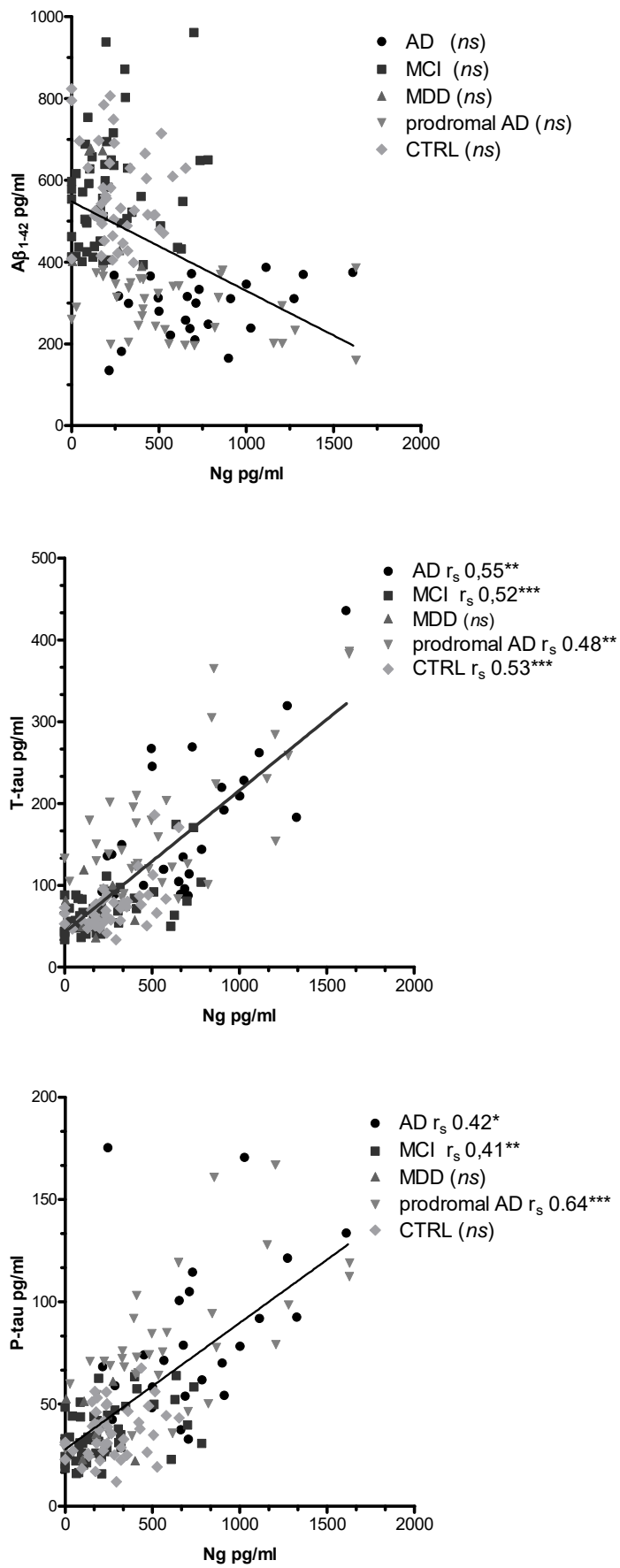
Fig. 1 Correlations between Ng and CSF biomarkers; *p<0.05; **p<0.01; ***p<0.001.

I would suggest that you make three panels, one for T-tau, one for P-tau and the last one for Abeta1-42. It would be great if AD, prodromal AD, non-AD MCI, controls and MDD patients were indicated by different symbols and if the p-values in each diagnostic group were given in the figure legend.

Table 2. Correlation between Ng, CSF biomarkers and cognitive evaluation scales.

Rho Spearman	AD (n=25)	MCI (n=50)	Prodromal AD (n=36)	MDD (n=6)	CTRL (n=44)	All Group (n=161)
Ng vs $A\beta_{1-42}$	0.29	0.21	-0.24	-0.25	-0.13	-0,477***
Ng vs **T-tau**	0.55**	0.52***	0.48**	-0.02	0.52***	0,686***
Ng vs **P-tau**	0.43*	0.42**	0.64***	0.25	0.19	0,607***
Ng vs **MMSE**	-0.33	0.14	0.18	0.06	-0.18	-0,244**
Ng vs **CAMCOG**	-0.45*	0.26	0.14	0.54	-0.08	-0,233**

*p<0.05; **p<0.01; ***p<0.001.

I follow your instructions… "Actually, when looking through these figures, I actually think you should simply show CSF Ng with T-tau, P-tau and Abeta42 correlations and put the correlation coefficients and p-values with cognitive scores into one table where you might also be able to include the correlation coefficients within diagnostic groups separately (or mention in the text that they were not significant)."

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