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ORIGINAL ARTICLE

Association of cerebrospinal fluid Neurogranin with Alzheimer's disease

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Abstract

Cerebrospinal fluid (CSF) Neurogranin has recently been proposed as a potential biomarker for cognitive decline and brain injury in Alzheimer's disease (AD). To test whether CSF Neurogranin levels are increased in AD and its association with cognitive decline, we examined 99 cognitively normal (CN) subjects, 171 patients with mild cognitive impairment (MCI), and 81 patients with AD in the cross-sectional study from the Alzheimer's Disease Neuroimaging Initiative (ADNI). The results showed that CSF Neurogranin was increased in both AD and MCI compared with controls. CSF Neurogranin was particularly high in patients with MCI and AD dementia with Aβ pathologic features. Neurogranin levels were significantly higher in females compared to males with MCI. Levels of Neurogranin between the males and females with AD and CN did not differ. Neurogranin levels were significantly higher in APOE ε4 carriers compared to APOE ε4 non-carriers with MCI. Levels of Neurogranin between the APOE ε4 carriers and APOE ε4 non-carriers with AD and CN did not differ. Elevated CSF Neurogranin levels were positively correlated with levels of total tau and P-tau in AD. The results indicated that CSF Neurogranin was increased at the prodromal stage of AD and might reflect synaptic injury as cognitive decline in AD.

Keywords Alzheimer's disease · Neurogranin · Cerebrospinal fluid · Mild cognitive impairment

Introduction

Alzheimer's disease (AD) is treated as the most prevalent neurodegenerative disorder worldwide. Recently, synaptic dysfunction and degeneration has been considered as an early pathogenic event in the progression of AD, and has a stronger association with cognitive impairment compared with plaque or tangles [1–5]. Thus, synaptic biomarkers may serve as promising tools to detect synaptic dysfunction in the progression of AD. Neurogranin, a postsynaptic protein enriched in dendritic spines within the hippocampus, amygdala and cerebral cortex [6], is a putative marker of synaptic loss in AD [7]. A recent study revealed that cerebrospinal fluid (CSF) Neurogranin expression was already significantly higher in patients with AD compared to other dementias [8, 9] in early clinical stages [10, 11]. In the present study, we aimed to analyze CSF Neurogranin concentration in cognitively normal (CN) individuals, patients with mild cognitive impairment (MCI), and patients with AD dementia, whose physiological and pathological data were collected and shared publicly in the Alzheimer's Disease Neuroimaging Initiative (ADNI) study platform, to investigate whether Neurogranin could work as a potential biological marker for synaptic loss to reflect ongoing cognitive decline.

Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (https://adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

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Materials and methods

ADNI study design

Data used in the preparation of this article were obtained from the ADNI database (https://adni.loni.usc.edu). The ADNI was launched in 2003 as a public–private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). The Principal Investigator of this initiative is Michael W. Weiner, MD, VA Medical Center and University of California-San Francisco. ADNI is a global research effort that actively supports the investigation and development of treatments that slow or stop the progression of AD and subjects have been recruited from over 50 sites across the US and Canada. The overall goal of ADNI is to determine biomarkers for use in Alzheimer's disease clinical treatment trials. To date, it has three phases: ADNI1, ADNI GO and ADNI2, consisting of cognitively normal (CN) individuals, early mild cognitive impairment (EMCI), to late mild cognitive impairment (LMCI), and dementia or AD. For more information, see http://www.adni-info.org. Institutional review board approval was conducted at each ADNI site. Written informed consent was obtained from all participants or authorized representative.

Subjects

We included all CN, MCI and AD dementia subjects with available baseline CSF Neurogranin, Aβ42, total tau, and P-tau from ADNI-1. Inclusion/exclusion criteria are described in detail at http://www.adni-info.org. Briefly, all subjects included were between the ages of 55 and 90 years, had completed at least 6 years of education, were fluent in Spanish or English, and were free of any significant neurological disease other than Alzheimer's disease. CN had Mini-Mental State Examination (MMSE) score ≥ 24 and clinical dementia rating (CDR) score 0. MCI subjects had MMSE score ≥ 24, objective memory loss as shown on scores on delayed recall of the Wechsler memory scale logical memory II [> 1 standard deviations (SD) below the normal mean], CDR 0.5, preserved activities of daily living, and the absence of dementia.

AD dementia patients fulfilled the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria for probable AD [12] and had MMSE 20-26 and CDR 0.5 or 1.0. Totally, 351 individuals (99 participants with CN, 171 participants with MCI, and 81 participants with AD) were included in the present study.

Cognitive functions

Global cognition was assessed by MMSE [13], Alzheimer's disease assessment scale-cognitive subscale (ADAS-Cog) [14] and global clinical dementia rating scale (CDR-SB) at baseline.

Detection of Neurogranin in CSF

CSF procedures have been described previously [15]. The levels of CSF Neurogranin were determined at the Clinical Neurochemistry Laboratory, University of Gothenburg, Sweden, and are available in the ADNI database. CSF Neurogranin was analyzed by electrochemiluminescence technology (Meso Scale Discovery, Gaithersburg, Maryland, USA) using Neurogranin7, which is a monoclonal antibody specific for Neurogranin, as coating antibody and polyclonal Neurogranin anti-rabbit (ab 23570, Upstate) as detector antibody [11]. Values are given as pg/mL.

Detection of CSF Aβ42, total tau, and P-tau

CSF Aβ42, Total tau, and P-tau were measured at the ADNI biomarker core (University of Pennsylvania) using the multiplex xMAP Luminex platform (Luminex Corp, Austin, TX, USA) with the INNOBIA AlzBio3 kit (Fujirebio, Ghent, Belgium) in previous publication [15–17]. Values are given in pg/mL for both tau and Aβ42. Subjects were dichotomized into Aβ positive or Aβ negative using the previously established cut-off (CSF Aβ42 < 192 pg/mL) [15].

Apolipoprotein E genotyping

APOE (gene map locus 19q13.2) genotypes of the study subjects were obtained from the ADNI database (https://adni.loni.usc.edu).

Statistical analysis

Demographic data were compared among the CN, MCI and AD groups using one-way ANOVA for continuous variables by mean ± standard deviation (SD), Chi-square test for the frequencies of categorical variables (such as gender, genotype distribution), Kruskal–Wallis test for skewed distribution variables by median (M) and interquartile range (IQR). Mann–Whitney U test was used to examine the CSF Neurogranin by gender and APOE ε4 carriers in the three groups.

The Spearman's correlation test was applied to analyze the association between CSF Neurogranin and age, education, MMSE, CSF Aβ42, total tau and P-tau in the whole sample and within each diagnostic group. All statistics were performed using SPSS software (version 23.0; IBM SPSS). All calculated tests were two-sided, and the level of statistical significance was set at $P < 0.05$. Figures were produced using GraphPad Prism 6.

Results

Demographic characteristics of the subjects

The demographic and clinical information of the study subjects are shown in Table 1.

There were no statistically significant differences in age and education across the three groups. The MCI group had fewer females than the other two study groups ($P = 0.027$). The APOE ε4 carriers in AD, MCI, CN were 70.4, 52, 24.2% ($P = 0.000$). In addition, significant differences in MMSE, ADAS-Cog, CDR-SB scores were detected across the three groups (AD < MCI < CN, 24 vs. 27 vs. 29, $P = 0.000$; AD > MCI > CN, 17.7 vs. 11.3 vs. 6.3, $P = 0.000$; AD > MCI > CN, 4 vs. 1.5 vs. 0.0, $P = 0.000$). There were statistically significant differences in CSF Aβ42, total tau, P-tau and Neurogranin across the three groups ($P = 0.000$, $P = 0.000$, $P = 0.000$, $P = 0.000$). Consistent with previous findings, the subjects with AD had the lowest CSF Aβ42 and the highest CSF total tau and P-tau protein [18].

Levels of CSF Neurogranin in different diagnostic groups

CSF Neurogranin levels were significantly elevated in AD and MCI subjects compared with CN subjects (median, AD > MCI > CN, 471 vs. 455 vs. 324 pg/mL, $P = 0.000$, $P = 0.001$, Table 1; Fig. 1).

Levels of CSF Neurogranin and Aβ Pathologic Features

We compared CSF Neurogranin between Aβ-negative controls, Aβ-positive controls, Aβ-negative patients with MCI, Aβ-positive patients with MCI, Aβ-negative patients with AD dementia and Aβ-positive patients with AD dementia

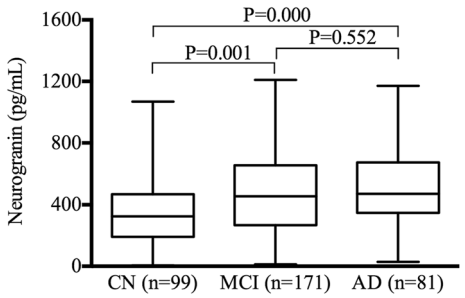


Fig. 1 CSF Neurogranin levels in different diagnostic groups. Boxplots showing CSF Neurogranin concentrations in CN, MCI and AD. The data are presented as median (M) and interquartile range (IQR). A significant difference in CSF Neurogranin levels was found across the three groups (median, AD > MCI > CN, 471 vs. 455 vs. 324 pg/mL), *P* values tested by Kruskal–Wallis test

Table 1 Demographic and clinical characteristics of subjects included in the study

Characteristics	CN ($n = 99$)	MCI ($n = 171$)	AD ($n = 81$)	*P* value
Age mean (SD)	75.5 (5.3)	74.2 (7.6)	74.6 (7.8)	0.625
Female [n (%)]	49 (49.5)	58 (33.9)	37 (45.7)	0.027
APOE ε4 [n (%)]	24 (24.2)	89 (52.0)	57 (70.4)	0.000
Education	16 (14–18)	16 (14–18)	16 (12–18)	0.259
MMSE	29 (29–30)	27 (25–29)	24 (22–25)	0.000
ADAS-cog	6.3 (4.0-8.3)	11.3 (8.7–14.3)	17.7 (13.8–21.3)	0.000
CDR-SB	0.0 (0.0–0.0)	1.5 (1.0–2.0)	4.0 (3.5-5.0)	0.000
CSF Aβ42	221 (158–258)	146 (128–208)	137 (122–162)	0.000
Aβ+ [n (%)]	37 (37.4)	126 (73.7)	74 (91.4)	0.000
CSF total tau	65 (51–89)	87 (65–118)	111 (82–145)	0.000
CSF P-tau	21 (16–30)	32 (22–45)	35 (29–47)	0.000
CSF Neurogranin	324 (191–468)	455 (267–657)	471 (347–675)	0.000

CN cognitively normal, *MCI* mild cognitive impairment, *AD* Alzheimer disease, *MMSE* Mini-Mental State Examination, *ADAS-cog* Alzheimer's disease assessment scale-cognitive subscale, *CDR-SB* global clinical dementia rating scale, *CSF* cerebrospinal fluid, P-tau phosphorylated tau

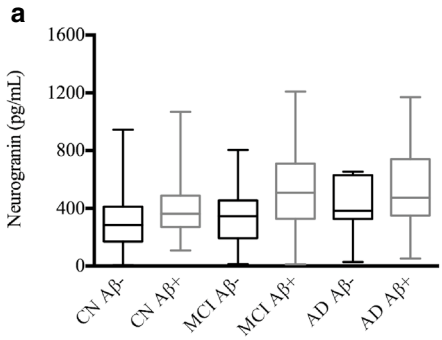
Aβ positivity was defined as CSF Aβ42 less than 192 pg/mL. Values are expressed in pg/mL. Values were described as median (M) and the interquartile range (IQR) unless otherwise specified. *P* values tested by One-Way ANOVA, Chi square test and Kruskal–Wallis test

(Fig. 2). The Aβ-positive patients with AD dementia group had higher CSF Neurogranin than Aβ-negative CN (median, 475 vs. 284 pg/mL, $P=0.000$), Aβ-negative MCI (median, 475 vs. 347 pg/mL, $P=0.002$), Aβ-positive CN (median, 475 vs. 363 pg/mL, $P=0.020$). Furthermore, Aβ-positive MCI had increased CSF Neurogranin compared to Aβ-negative CN (median, 510 vs. 284 pg/mL, $P=0.000$), Aβ-negative MCI (median, 510 vs. 347 pg/mL, $P=0.001$), Aβ-positive CN (median, 510 vs. 363 pg/mL, $P=0.016$). There were no statistically significant differences between Aβ-negative and Aβ-positive controls and Aβ-negative patients with MCI.

Levels of CSF Neurogranin and clinical characteristics

The CSF Neurogranin on the subjects by gender is showed in Fig. 3. CSF Neurogranin levels were increased in females in MCI group ($P=0.000$), while did not differ by gender in AD and CN groups ($P=0.763$, $P=0.293$).

Furthermore, to explore the association of the APOE ε4 genotype with CSF Neurogranin, CSF Neurogranin levels were compared between APOE ε4 carriers and non-carriers in the three groups. As shown in Fig. 4, the results suggested



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	MCI Aβ+	AD Aβ+
CN Aβ-	0.000	0.000
MCI Aβ-	0.001	0.002
CN Aβ+	0.016	0.020

Fig. 2 CSF Neurogranin concentrations in different combinations of clinical diagnosis and Aβ pathology. Box-plots displaying CSF Neurogranin concentrations in CN, MCI and AD groups, stratified by occurrence of Aβ positivity (CSF Aβ42 < 192 pg/mL) (CN Aβ-, $n=62$, CN Aβ+, $n=37$, MCI Aβ-, $n=45$, MCI Aβ+, $n=126$, AD Aβ-, $n=7$, AD Aβ+, $n=74$). The data are presented as median (M) and interquartile range (IQR). Values are expressed in pg/mL. *NS* not significant ($P>0.05$). *P* values tested by Kruskal–Wallis test

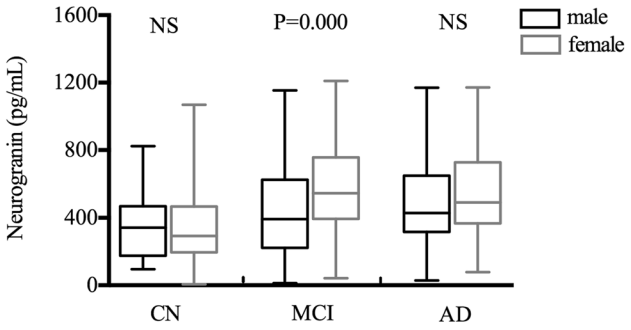

Fig. 3 Comparison of CSF Neurogranin in males and females with CN, MCI and AD. Box-plots displaying CSF Neurogranin levels in CN, MCI and AD groups. In the MCI group, the female had significantly higher levels of CSF Neurogranin compared to male ($P=0.000$). Values are expressed in pg/mL. *NS* not significant ($P>0.05$). *P* values tested by Mann–Whitney test

that the levels of CSF Neurogranin were statistically significantly elevated in the subjects with MCI who carry the APOE ε4 allele ($P=0.000$). The APOE ε4 carriers tend to demonstrate higher CSF Neurogranin compared with APOE ε4 non-carriers but represent no statistical difference ($P=0.901$, $P=0.391$).

Correlation of Neurogranin with age, education, MMSE score, CSF Aβ42, total tau, and P-tau

Spearman correlation analyses were performed to examine the relationships between CSF Neurogranin levels and clinical characteristics and other CSF biomarkers in all samples and within each diagnostic group (Table 2). The correlation between CSF Neurogranin and total tau, P-tau was especially strong in all samples and within each diagnostic group ($R=0.751$, $P=0.000$; $R=0.657$, $P=0.000$; $R=0.721$,

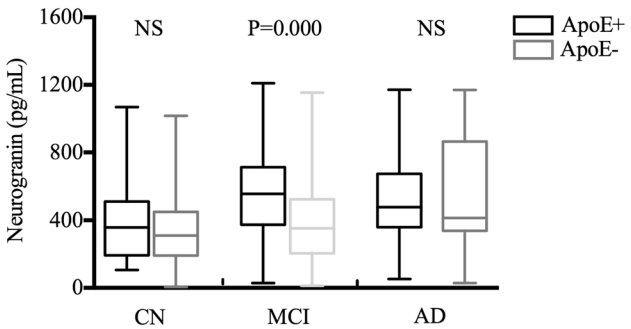

Fig. 4 Comparison of CSF Neurogranin levels in APOE ε4 carriers and APOE ε4 non-carriers with CN, MCI and AD. Box-plots showing CSF Neurogranin levels in CN, MCI and AD groups. CSF Neurogranin levels are significantly higher in APOE ε4 carriers with MCI than in APOE ε4 non-carriers ($P=0.000$). Values are expressed in pg/mL. *NS* not significant ($P>0.05$). *P* values tested by Mann–Whitney test

Table 2 Correlations of CSF Neurogranin levels with clinical variables and core CSF biomarkers

Group	All subjects ($n=351$)		CN ($n=99$)		MCI ($n=171$)		AD ($n=81$)	
	R	*P*	*R*	*P*	*R*	*P*	*R*	*P*
Age	− 0.114*	0.032	0.142	0.162	− 0.128	0.094	− 0.293	0.008
Education	− 0.095	0.074	0.000	0.998	− 0.102	0.185	− 0.154	0.170
MMSE	− 0.159**	0.003	0.042	0.679	0.061	0.429	− 0.021	0.855
CSF Aββ42	− 0.307**	0.000	− 0.163	0.108	− 0.296**	0.000	− 0.189	0.091
CSF total tau	0.751**	0.000	0.721**	0.000	0.739**	0.000	0.690**	0.000
CSF P-tau	0.657**	0.000	0.558**	0.000	0.622**	0.000	0.708**	0.000

Associations were investigated by Spearman's correlation analyses. The correlation coefficient is expressed as R

* $P < 0.05$

** $P < 0.01$

$P=0.000$; $R=0.558$, $P=0.000$; $R=0.739$, $P=0.000$; $R=0.622$, $P=0.000$; $R=0.690$, $P=0.000$; $R=0.708$, $P=0.000$). A negative relationship between CSF Neurogranin level and CSF Aβ42 was found in all subjects and MCI group ($R=-0.307$, $P=0.000$; $R=-0.296$, $P=0.000$). An inverse correlation between CSF Neurogranin level and age was observed in the whole sample ($R=-0.114$, $P=0.032$). A negative correlation between CSF Neurogranin level and MMSE was found in all subjects ($R=-0.159$, $P=0.003$). In addition, there was no significant correlation between CSF Neurogranin level and education in entire sample and within each group ($R=-0.095$, $P=0.074$; $R=0.000$, $P=0.998$; $R=-0.102$, $P=0.185$; $R=-0.154$, $P=0.170$).

Discussion

Neurogranin is a neural-specific postsynaptic protein involved in long-term potentiation (LTP) signaling and memory consolidation [6, 11, 19, 20]. It is highly expressed in neuronal cell bodies and dendritic spines of cerebral cortex, hippocampus and basal forebrain by excitatory neurons. In other words, it is the same brain regions where regulate the availability of calmodulin [6, 21–23] that are affected in AD [24, 25].

In the present study, we evaluated the performance of CSF Neurogranin as a novel diagnostic biomarker for synaptic pathology in the prodromal and dementia stages of AD.

The major findings of the study were that CSF Neurogranin levels demonstrated statistical higher values in AD dementia group and MCI group in comparison with CN group, suggesting that increased CSF Neurogranin concentrations may present a critical feature in the pathogenesis of AD. This result is consistent with the previous studies of Kvartsberg and Portelius [7, 11].

To examine if CSF Neurogranin levels could be utilized to identify MCI patients with an underlying Aβ pathology, each group involved in the research was divided into either Aβ-positive (Aβ < 192 pg/mL) or Aβ-negative (Aβ > 192 pg/mL). As was expected, the CSF Neurogranin levels were statistically significantly higher in the CSF Aβ-positive AD group. In addition, high CSF Neurogranin levels were also reported in the Aβ-positive MCI group, indicating that CSF Neurogranin is an early pathophysiological marker of AD-related synaptic damage, which is consistent with other recent findings on Neurogranin in other studies, and is further supported by observations that high CSF Neurogranin associated with a faster rate of cognitive deterioration in MCI subjects [10, 26, 27].

Regarding to gender, a statistical stronger relationship was found between APOE ε4 effect on CSF Neurogranin levels and females compared to that with males. Statistical analysis suggests that a higher CSF Neurogranin levels, combined with other reported metabolic and structural changes in female APOE ε4 carriers, may lead to a higher risk of AD in females [28–30].

A statistically significant difference was found in comparison of CSF Neurogranin levels in APOE ε4 carriers vs. APOE ε4 non-carriers with MCI. Data analysis also demonstrated higher CSF Neurogranin levels in CN and AD APOE ε4 carriers compared to non-carriers, though no statistical significance was discovered between the two groups. Previous studies demonstrated that postsynaptic function modulation could be achieved by APOE ε4 [31–33].

Last but not the least, CSF Neurogranin concentrations were found to be significantly associated with CSF total tau and P-tau levels in the whole sample and diagnostic groups. High CSF Neurogranin levels were found to be related with low CSF Aβ42 and high CSF total tau and P-tau in MCI. This relationship gives support to the utilization of CSF Neurogranin as a biomarker, which is sensitive to AD-related biological changes in prodromal AD. There is evidence that tau pathology is involved in synapse degeneration and contributes to cognitive decline [34, 35]. The absence of correlation between CSF Neurogranin level and Aβ42 level in AD is in agreement with the studies, showing that

there were no correlations between both the synapse loss and clinical stage and the amount of amyloid plaques [2, 36, 37].

Taken together, these findings support that CSF Neurogranin could be used as a potential biomarker in the cascade of neural events leading to AD.

A few limitations should be addressed in the study. First, the cross-sectional design used in the study does not allow to assess the potential changes of Neurogranin levels over time. Future studies are needed to confirm our conclusions based on longitudinal population. Second, the restricted sample selection in the ADNI should be taken into consideration for interpreting the data.

Conclusion

The results indicate that CSF Neurogranin concentration is related to AD-characteristic pathophysiology process. Further studies are needed to explore the diagnostic value of CSF neurogranin.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in the study involving human participant were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed Consent Written informed consent was obtained from all participants or authorized representative.

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