

Association of Clusterin Levels in Cerebrospinal Fluid with Synaptic Degeneration Across the Alzheimer's Disease Continuum

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Purpose: Although emerging evidence has suggested that clusterin is involved in the pathogenesis of Alzheimer's disease (AD), the association of clusterin with synaptic degeneration in living human is unclear. In the present study, we aimed to examine the association of CSF clusterin levels with synaptic degeneration in individuals with different severities of cognitive impairment.

Patients and Methods: In the present study, we compared levels of clusterin in CSF among individuals with normal cognition (NC), mild cognitive impairment (MCI), and AD. Further, linear regression models were performed to examine the association of CSF clusterin with neurogranin (NG, reflecting synaptic degeneration) with adjustment of several potential confounders.

Results: We found that CSF clusterin levels were positively correlated with NG in the NC and MCI groups, but not the AD group. In all subjects, linear regression models suggested that clusterin levels were positively associated with NG levels independent of age, gender, apolipoprotein E4 (APOE4) genotype, clinical diagnosis, and CSF A β 42 levels.

Conclusion: Our data indicated that clusterin was associated with CSF NG levels among older individuals with different severities of cognitive impairment.

Keywords: clusterin, neurogranin, synaptic degeneration, Alzheimer's disease, mild cognitive impairment

Introduction

Clusterin, also known as apolipoprotein J, is involved in the pathogenesis of Alzheimer's disease (AD).¹ Previous studies showed that clusterin levels were significantly elevated in cerebrospinal fluid (CSF) and brain of AD patients, and clusterin levels in plasma were found to be related with disease severity and brain atrophy in AD patients.^{2,3} It has been suggested that clusterin could interact with β -amyloid (A β) and facilitate its clearance from brain.^{4,5} Further, it has also been reported that clusterin could interact with A β to form a stable complex⁶⁻⁹ and enhance its clearance from brain across the blood-brain barrier (BBB) via low-density lipoprotein receptor-related protein-1 (LRP1).⁶ However, the exact mechanisms by which clusterin contributes to the pathogenesis of AD remain elusive.

It has been reported that synaptic degeneration is an important mechanism underlying cognitive deficit in AD.¹⁰ Neurogranin (NG), a postsynaptic protein, has been reported to be significantly increased in the CSF of patients with mild cognitive impairment (MCI) and AD.¹¹⁻¹³ In addition, CSF NG levels can predict

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progression of MCI to AD dementia.¹⁴ In animals, knock-down of NG inhibits long-term potentiation (LTP) and cognition,¹⁵ while upregulation enhances LTP and cognition.¹⁶ Given the potential roles of both clusterin and NG in AD pathogenesis, we hypothesized that clusterin may contribute to synaptic dysfunction, which leads to cognitive deficits in AD.

First, we compared CSF clusterin levels among individuals with normal cognition (NC), MCI, and AD. Second, to examine the relationship between CSF clusterin and NG levels, the Pearson correlation test was conducted in each diagnostic group. Finally, linear regression models were performed to examine the association of CSF clusterin with NG levels by controlling for age, gender, educational attainment, APOE4 genotype, clinical diagnosis, and CSF A β 42 levels.

Patients and Methods

Alzheimer's Disease Neuroimaging Initiative (ADNI)

Cross-sectional data used in the preparation of this paper were extracted from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database. The primary aim of ADNI has been to examine whether demographics, cognitive assessments, serial MRI, PET, blood, and CSF biomarkers could be integrated to predict the progression of MCI and AD. This study was approved by local ethical committees. All participants provided written informed consent. The official or affiliated names of the approving local ethics committee is available in the [Supplementary document](#).

Participants

We selected subjects who met criteria for mild AD, MCI and NC and had CSF clusterin and NG samples.

Subjects with NC had a Mini-Mental State Examination (MMSE)¹⁷ score ranging from 24 to 30 and a Clinical Dementia Rating (CDR)¹⁸ score of 0. Individuals with MCI had a MMSE score ranging between 24 and 30, a CDR score of 0.5, an objective memory decline as evidenced by the Wechsler Memory Scale Logical memory II, and an absence of dementia. In the ADNI study, the type of MCI participants was amnesic MCI. Patients with mild AD 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 dementia¹⁹ and had a MMSE score ranging between 20 and 26 and a CDR score ranging between 0.5 and 1.

Measurement of Clusterin Levels in CSF

Clusterin levels in CSF were measured using Rules Based Medicine (Human Discovery MAP, v1.0),²⁰ details of which can be found at the ADNI website (<http://www.adni-info.org>). The analyte was log-transformed for statistical analyses. Values are given as ug/mL. The average of the coefficient of variation (CV) of clusterin levels was 9.63%.

Measurement of NG Levels in CSF

NG levels in CSF were determined by electrochemiluminescence technology (Meso Scale Discovery, Maryland, USA) with NG7 (a monoclonal antibody specific for NG)²¹ as the coating antibody and polyclonal NG anti-rabbit (ab 23570, Upstate) as the detector antibody¹¹. Values are given as pg/mL.

Measurement of CSF A β 42, t-tau, and p-tau Levels

CSF A β 42, t-tau, and p-tau levels were measured by the multiplex xMAP Luminex platform (Luminex Corp, Austin, TX), details of which have been described elsewhere²² and can be found at the ADNI website (<http://www.adni-info.org>). Values are given as pg/mL.

Statistical Analysis

F-tests and χ^2 tests were utilized to examine differences in demographics and clinical variables between the three diagnostic groups. The Pearson correlation analysis was applied to examine the relationship between clusterin and NG levels in CSF in each diagnostic group. Then, univariate analyses of predictors for CSF NG levels were conducted. Further, forward stepwise regression models were performed to examine the relationships between CSF clusterin and NG levels. The Akaike information criterion (AIC) was utilized to select the best model. All statistical analyses were conducted using R software (version 3.5.1). The level of statistical significance was set at $P < 0.05$.

Results

Demographic and Clinical Information on Study Participants

In the present study, there was a total of 294 individuals (84 individuals with NC, 143 individuals with MCI, and 67 patients with mild AD). There were significant differences in several variables between the three diagnostic groups ([Table 1](#)).

Table 1 Demographic and Clinical Characteristics and Protein Levels

Variables	Controls (n = 84)	MCI (n = 143)	AD (n = 67)	P values
Age, y	75.7 ± 5.53	74.7 ± 7.29	75.1 ± 7.64	0.56
Female, %	51.2	32.9 ^a	44.8	0.02
Education, y	15.7 ± 2.98	15.9 ± 2.95	15.1 ± 3	0.2
APOE4 carriers, %	23.8	53.8	70.1	< 0.001
MMSE scores	29.1 ± 0.9	26.9 ± 1.8 ^a	23.5 ± 1.9 ^{b, c}	< 0.001
CSF Aβ42, pg/mL	207 ± 53.8	160 ± 49.6 ^a	141 ± 35.5 ^{b, c}	< 0.001
CSF NG, pg/mL	366 ± 206	507 ± 304 ^a	553 ± 315 ^b	< 0.001
CSF clusterin, ug/mL	1.4 ± 0.15	1.4 ± 0.16	1.41 ± 0.18	0.86

Notes: ^aCN vs MCI, $p < 0.05$; ^bCN vs AD, $p < 0.05$; ^cMCI vs AD, $p < 0.05$.

Abbreviations: MCI, mild cognitive impairment; AD, Alzheimer's disease; MMSE, mini-mental state examination; Aβ42, β-amyloid 42; NG, neurogranin.

CSF Clusterin and NG Levels in the Three Diagnostic Groups

No significant difference was observed in CSF clusterin levels between the three diagnostic groups (Table 1 and Figure 1A). However, there was a significant difference in CSF NG levels between the three groups (Table 1 and Figure 1B).

Associations of CSF Clusterin with CSF NG and AD Pathologies in All Subjects

To examine the associations of CSF clusterin with CSF NG and AD pathologies, the Pearson correlation tests were applied

in the overall sample. As shown in Figure 2, we found that CSF clusterin levels were positively correlated with NG levels ($r = 0.22$, $p < 0.001$), t -tau ($r = 0.29$, $p < 0.001$) and p -tau ($r = 0.12$, $p = 0.04$), but not Aβ42 levels ($r = 0.06$, $p = 0.288$).

Correlations Between the Various Protein Levels and MMSE Scores in the Whole Sample

In order to examine the correlations between the various protein levels in CSF (clusterin, NG, and Aβ42) and MMSE scores in the whole sample, Spearman correlation

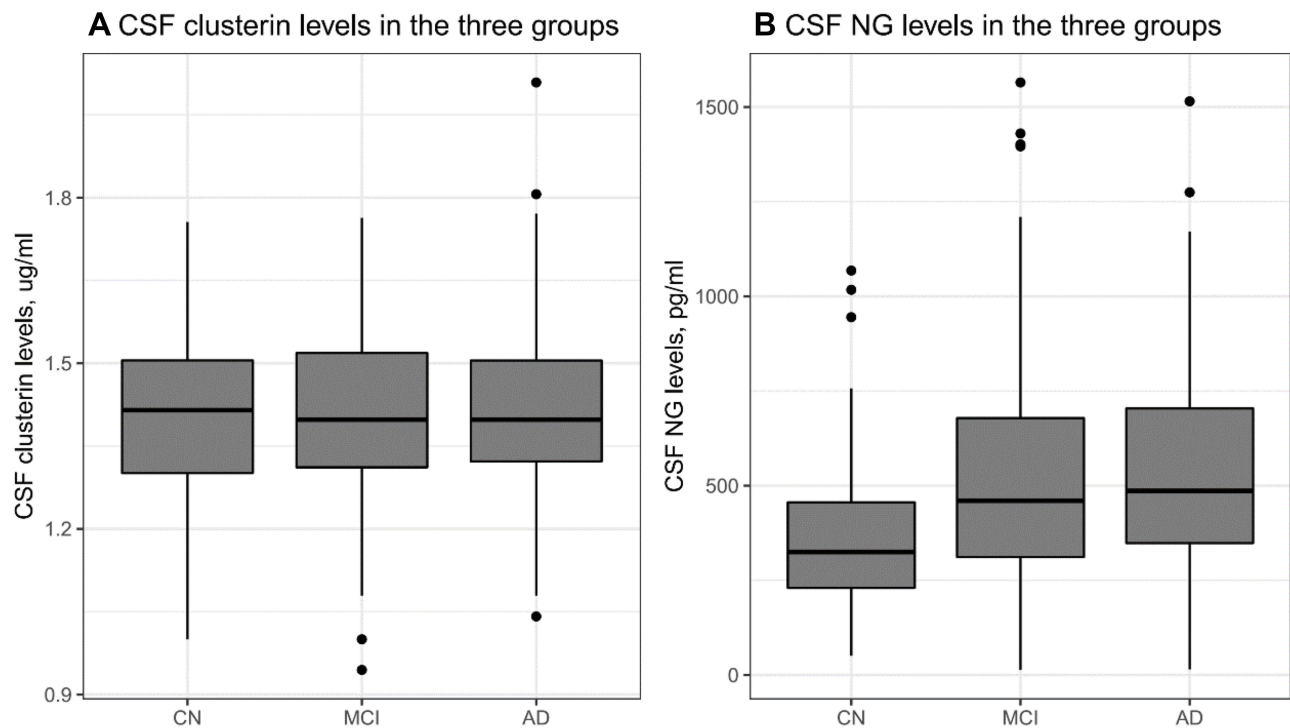


Figure 1 CSF clusterin and NG levels in the three diagnostic groups. (A) CSF clusterin levels were similar across subjects. (B) There was a significant difference in CSF NG levels across subjects.

Abbreviations: CN, cognitively normal; MCI, mild cognitive impairment; AD, Alzheimer's disease; NG, neurogranin.

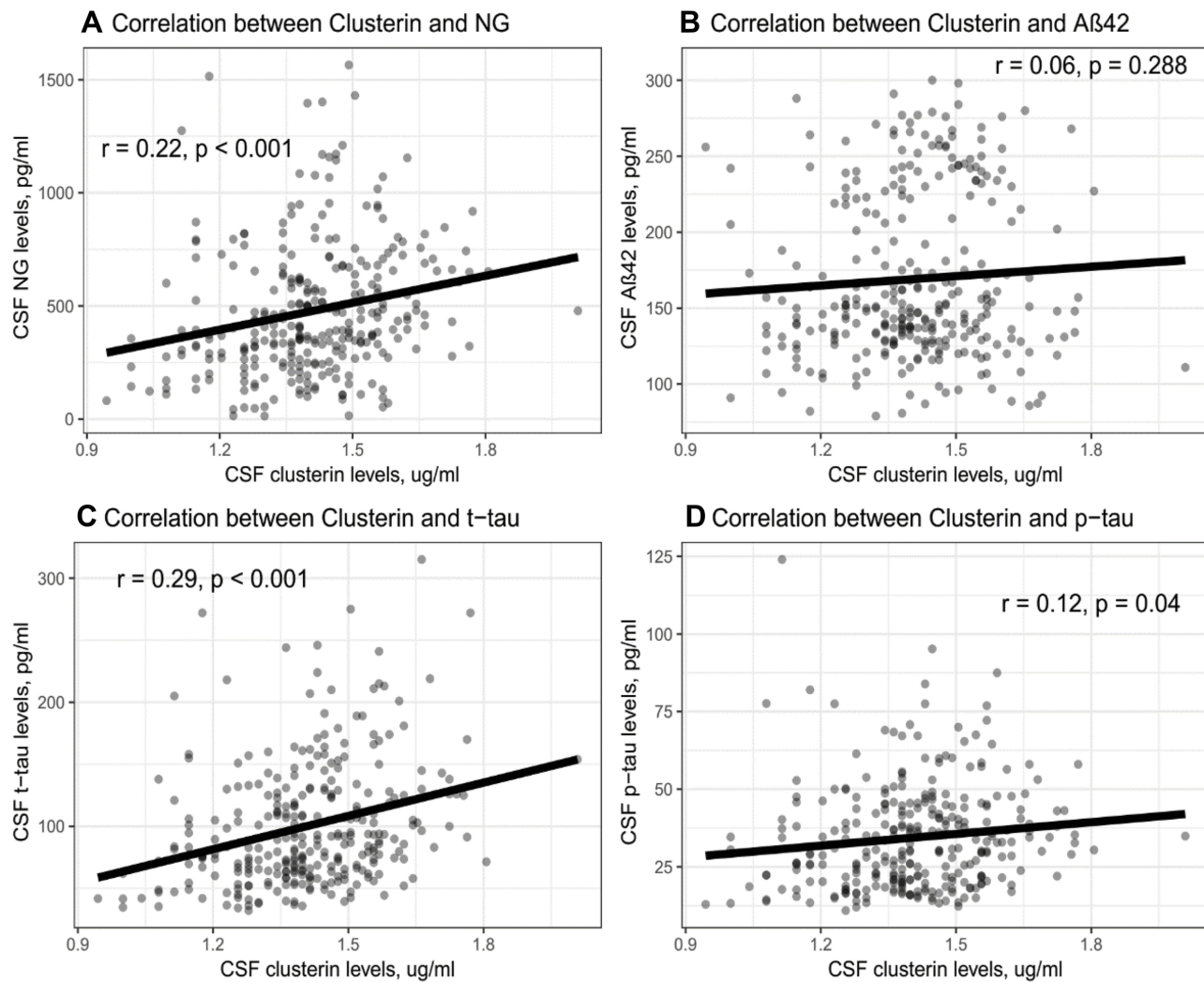


Figure 2 (A–D) Associations of CSF clusterin levels with NG levels and AD pathologies. We found that CSF clusterin levels were positively correlated with NG levels ($r = 0.22$, $p < 0.001$), t-tau ($r = 0.29$, $p < 0.001$) and p-tau ($r = 0.12$, $p = 0.04$), but not A β 42 levels ($r = 0.06$, $p = 0.288$).
Abbreviations: A β 42, β -amyloid 42; NG, neurogranin.

tests were performed in the whole sample. We found that MMSE scores were associated with CSF NG ($\rho = -0.19$, $p = 0.001$) and A β 42 levels ($\rho = 0.35$, $p < 0.001$), but not CSF clusterin levels ($\rho = 0.03$, $p = 0.66$).

Correlations Between CSF Clusterin and NG Levels in Each Diagnostic Group

To examine whether the relationship between CSF clusterin and NG levels was modified by cognitive status, Pearson's correlation tests were utilized to examine the correlation between CSF clusterin and NG levels in each diagnostic group. Interestingly, we found that CSF clusterin levels were associated with NG levels in the CN ($r = 0.46$, $p < 0.001$) and MCI ($r = 0.23$, $p = 0.005$)

groups, but not the AD group ($r = 0.046$, $p = 0.71$; Figure 3).

Association of CSF Clusterin with NG Levels

First, univariate analyses were conducted to systematically examine the relationships between each individual predictor and CSF NG levels. The results are demonstrated in Table 2. Second, to further examine the association of clusterin with NG levels in CSF, forward stepwise regression models were performed, and AIC was used to select the best fit. We found that CSF clusterin levels were associated with NG levels in CSF after adjusting for age, gender, APOE4 genotype, clinical diagnosis, and CSF A β 42 levels (Table 3).

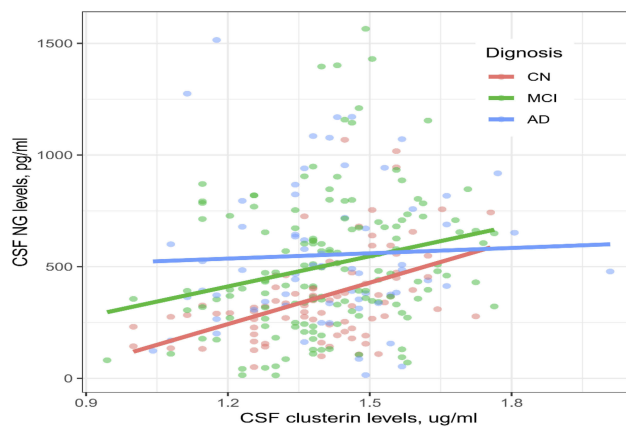


Figure 3 Correlation between CSF clusterin and NG levels in each diagnostic group.

Abbreviations: CN, cognitively normal; MCI, mild cognitive impairment; AD, Alzheimer's disease; NG, neurogranin.

Discussion

To the best of our knowledge, this is the first study to report that CSF clusterin levels were positively associated with NG levels in CSF (reflecting synaptic degeneration) independent of age, gender, APOE4 genotype, clinical diagnosis, and CSF amyloid pathology. In addition, levels

Table 2 Univariate Analysis of Predictors for CSF NG Levels

Variables	Beta	SE	P values
Clusterin	397.44	101.81	< 0.001
Age	-5.661	2.441	0.0211
Male gender	-104.37	34.03	0.002
Education	-6.625	5.706	0.247
APOE4 genotype	172.88	32.45	< 0.001
Diagnosis-MCI	140.78	38.85	< 0.001
Diagnosis-AD	186.35	46.29	< 0.001
CSF A β 42	-1.686	0.3	< 0.001

Note: Beta is unstandardized beta.

Abbreviations: MCI, mild cognitive impairment; AD, Alzheimer's disease; A β 42, β -amyloid 42.

Table 3 Summary of Forward Stepwise Regression Model

Variables	Beta	SE	P values
Clusterin	550	96	< 0.001
Age	-6.37	2.24	0.005
Male gender	-141.2	31	< 0.001
APOE4 genotype	64.1	35.9	0.07
Diagnosis-MCI	78.6	38.3	0.04
Diagnosis-AD	80.9	46.5	0.08
CSF A β 42	-1.17	0.35	< 0.001

Notes: The adjusted R² of this model is 0.2445. Beta is unstandardized beta.

Abbreviations: MCI, mild cognitive impairment; AD, Alzheimer's disease; A β 42, β -amyloid 42.

of clusterin in CSF were positively correlated with NG levels in the CN and MCI groups, but not the AD group. However, no significant difference was observed in CSF clusterin levels between the three diagnostic groups.

Although clusterin gene has been regarded as a strong genetic risk factor for late-onset AD,^{23,24} the mechanism by which clusterin contributes to the pathogenesis of AD remains unclear. We provided in vivo evidence that clusterin may impair synaptic function to increase the risk of AD. To the best of our knowledge, this is the first study to examine the relationship between CSF clusterin and NG levels in living human. In line with these findings, a preclinical study found that in an in vitro model of ischemia, exogenous administration of recombinant clusterin induced a substantial worsening of synaptic damage, suggesting a deleterious role of clusterin in synaptic structure and function.²⁵ In clinical studies, structural magnetic resonance imaging (MRI) allows for the assessment of brain atrophy, including loss of synapses and neurons.^{26,27} A longitudinal prospective study showed a significant association of the clusterin *A β interaction with longitudinal entorhinal cortex atrophy in non-demented older adults,²⁸ indicating that clusterin may accelerate amyloid deposition and induce A β -associated neuronal and synaptic degeneration in the early stage of AD.²⁹⁻³² Increasing evidence has been suggested that β -amyloid-mediated synaptic loss is dependent on the upregulation of the NMDA receptor-mediated activity and cytoplasmic calcium ions, and this process contributes to dendritic spine loss.³³⁻³⁵ Recently, in non-demented older adults, Slot and colleagues found that CSF clusterin levels partially mediated the relationship between CSF ApoE levels and CSF tau levels. However, they did not find that CSF clusterin mediated the relationship between APOE4 genotype and CSF A β 42 levels.³⁶ In the present study, we found that CSF clusterin was associated with CSF NG levels after adjusting for CSF A β levels, indicating that this association may go beyond the effect of A β on synaptic function. However, further preclinical and clinical studies are needed to support this notion.

However, it is also likely that the upregulation of clusterin levels may represent a neuroprotective response. For instance, it has been reported that clusterin can reduce amyloid formation and provide significant cytoprotection.³⁷ In addition, clusterin has been reported to enhance amyloid clearance from brain across the BBB via LRP2.⁶ Further, a prospective longitudinal study conducted in Rotterdam found that clusterin levels in plasma were associated with the prevalence and severity of AD, but not the incidence

of AD during follow-up, suggesting that the upregulation of clusterin may be triggered by the neurodegenerative changes that occur in AD.²

Interestingly, we found that levels of clusterin in CSF were positively correlated with NG levels in the CN and MCI groups, but not the AD group. Our data highlighted a potential role of clusterin in the early stages of AD. However, further studies are warranted to examine the mechanism by which clusterin affects synaptic degeneration in the early stages of AD.

Several limitations should be noted. First, the cross-sectional design applied in the present study limits the ability to differentiate whether increased clusterin levels result from, cause, or are just correlated with synaptic degeneration among older individuals. Further preclinical and clinical studies are needed to examine the precise relationship between clusterin and synaptic degeneration. Second, CSF readout may not reflect what exactly happening in the brain. Therefore, recent advancements, such as multiplexed single synapse assay using SynTOF panel,³⁸ and more direct assessments of biomarkers in interstitial brain tissue³⁹ should be utilized in further studies. Third, the ADNI study represents a convenience sample of volunteers, which may reduce the power to generalize our findings to other populations. Further, most of the participants of the ADNI study are Caucasian and well educated, which may also limit the generalizability of our findings. However, the ADNI study is one of the most successful studies focusing on examining whether multiple biomarkers could be used to measure the progression of MCI and early AD dementia. More markers and more neurodegenerative diseases should be included in the ADNI study to further facilitate the biomarker discovery.

In conclusion, our data indicated that CSF clusterin was associated with CSF NG levels among older individuals with different severities of cognitive impairment.

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Disclosure

The authors declare that they have no conflict of interest.

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