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APOE ε4 carriers may undergo synaptic damage conferring risk of Alzheimer's disease

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Abstract

Introduction—Pathogenesis of Alzheimer's disease (AD) in apolipoprotein E ε4 (*APOE* ε4) carriers remains unclear. We hypothesize that *APOE* isoforms have differential effects on synaptic function.

Methods—We compared levels of CSF neurogranin (Ng) between *APOE* ε4 carriers and noncarriers in 399 subjects with normal cognition, mild cognitive impairment (MCI), and AD. We examined associations between Ng levels and age, education, gender, CSF-Aβ42, and tau protein.

Results—Neurogranin levels were significantly higher in *APOE* ε4 carriers compared to *APOE* ε4 noncarriers with MCI. Levels of Ng between the *APOE* ε4 carriers and *APOE* ε4 noncarriers with AD did not differ. Ng levels were correlated with MMSE and levels of tau and Aβ42.

Discussion—Significantly higher CSF Ng levels in *APOE* ε4 carriers with MCI may reflect synaptic injury underlying early cognitive impairment. Neurogranin may be an early biomarker of AD and important for disease diagnosis and timing of intervention in *APOE* ε4 carriers.

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jalz.2016.05.003.

Keywords

Synaptic function; Neurogranin; *APOE* ε4; Alzheimer's disease; *APOE*; Mild cognitive impairment

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia. Pathologically, it is characterized by extracellular amyloid deposition and intracellular accumulation of hyperphosphorylated tau protein in a patient's brain. In late-onset AD, over half of all AD cases are associated with the *APOE* ε4 genotype, highlighting the important role of *APOE* ε4 in AD pathogenesis [1].

APOE ε4 carriers with AD or amnestic mild cognitive impairment (MCI) have lower β-amyloid 42 (Aβ42), elevated total tau (*t*-tau), and phospho-tau (*p*-tau) in cerebrospinal fluid (CSF), compared to *APOE* ε4 noncarriers [2]. Structural magnetic resonance imaging (MRI) studies have shown that *APOE* ε4 carriers with AD and MCI have greater medial temporal lobe atrophy, particularly in the hippocampal area, compared to *APOE* ε4 noncarriers [3]. The accumulated evidence strongly supports the hypothesis that the *APOE* ε4 genotype exerts multiple effects on brain metabolism and structure.

Currently, the mechanism by which the *APOE* ε4 genotype contributes to the earlier onset and rapid progression of AD remains unclear. Although the *APOE* ε4 genotype affects amyloid metabolism, the association between amyloid deposition and cognitive impairment in AD is inconsistent. This suggests that other pathophysiological factors may be involved in cognitive decline seen among *APOE* ε4 carriers.

Synaptic dysfunction has been postulated as a central mechanism underlying cognitive impairment in AD [4]. Additionally, neuropathologic and biochemical studies demonstrate that postsynaptic components are damaged in AD [5]. The postsynaptic protein, drebrin, is remarkably reduced in the frontotemporal region of the AD brain, including in the hippocampus, compared to normal controls [6,7]. Neurogranin (Ng) is a postsynaptic protein which is highly expressed in hippocampus and involved in memory consolidation [8]. Kvartsberg's and Maartje's groups reported that Ng was markedly elevated in the CSF of patients with AD and MCI, indicating that Ng may be a biological marker reflecting synaptic integrity [9,10]. We thus hypothesized that *APOE* ε4 has detrimental effects on synaptic function, leading to elevated central nervous system Ng levels, which may in turn contribute to cognitive impairment in those *APOE* ε4 carriers who develop MCI and AD.

To investigate the effect of *APOE* ε4 on Ng, CSF Ng levels were examined in participants with normal cognition, MCI, and AD from the Alzheimer's Disease Neuroimaging Initiative (ADNI) dataset. We compared CSF Ng levels between *APOE* ε4 carriers and *APOE* ε4 noncarriers and examined the gene dose-effect of *APOE* ε4 on CSF Ng levels. We examined the correlation between Ng and mini-mental state examination (MMSE) score and levels of CSF Aβ42, *t*-tau protein, and *p*-tau protein. Finally, we analyzed the association of *APOE* ε4

with CSF Ng by controlling for age, education, gender, clinical diagnosis, and CSF levels of Aβ42, *t*-tau, and *p*-tau.

2. Materials and methods

2.1. ADNI study

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database. The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal has been to test whether serial MRI, positron emission tomography, other biological markers, and clinical and neuropsychological assessments can be combined to measure the progression of MCI and early AD. In our study, participants with an initial analysis of CSF Ng were included. Institutional review board approval was obtained at each ADNI site, and informed consent was obtained from each participant or authorized representative. Demographic information was extracted from the ADNI database. In this study, there were 111 participants with normal cognition, 193 participants with MCI, and 95 participants with AD.

2.2. CSF analyses

2.2.1. Quantification of Ng in CSF—The levels of CSF neurogranin (Ng) were determined at the Clinical Neurochemistry Laboratory, University of Gothenburg, Sweden, and are available in the ADNI database. A validation study of the Ng assay was performed [9]. CSF Ng levels of the ADNI samples were measured in duplicate. CSF Ng was analyzed by electrochemiluminescence technology (Meso Scale Discovery, Gaithersburg, Maryland, USA) using Ng 7, a monoclonal antibody specific for Ng as a coating antibody, and polyclonal Ng anti-rabbit (ab 23,570, Upstate) as a detector antibody [9]. Values are given in pg/mL.

2.2.2. Quantification of CSF Aβ42, t-tau, and p-tau—The CSF levels of Aβ42, *t*-tau, and *p*-tau were analysed by Leslie M. Shaw and John Q. Trojanowski's group, Department of Pathology & Laboratory Medicine and Center for Neurodegenerative Diseases Research, Perelman School of Medicine, University of Pennsylvania. The xMAP Luminex platform and Innogenetics/Fujirebio Alz-Bio3 immunoassay kits were used [11]. Linear regression analyses were performed for Aβ1–42 and *t*-tau to compare the CSF concentration results obtained. Values are given as pg/mL for both tau and Aβ42.

2.3. APOE allele genotyping

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

2.4. Statistical analysis

The F-test was used to examine the differences in continuous variables, and the χ^2 test was used to compare the frequencies of categorical variables among the control, MCI, and AD groups. For multiple comparisons of means, statistically significant results for individual variables were followed by post hoc pairwise comparisons with the Tukey's HSD (honest

significant difference) test and the Tukey-Kramer adjustment (unequal group sizes) [12]. The Pearson correlation test was used to analyze the correlation between CSF Ng and other variables. To examine the potential association between the *APOE* ε4 genotype and CSF Ng levels, several general linear regression models were constructed: model 1 was unadjusted; model 2 was adjusted for age, sex, and education attainment; model 3 was additionally adjusted for diagnostic status; and model 4 was additionally adjusted for tau and Aβ42 levels. All data analyses were performed with SAS statistical software version 9.3 package for Windows (SAS Institute, Cary, NC) [13]. The level of statistical significance was set at $P < .05$. In Table 1, values are expressed as mean ± standard deviation. In Fig. 1, the error bars represent standard deviation. In Fig. 2, the error bars represent 95% confidence intervals adjusted by age, education, and gender.

3. Results

3.1. Demographic information on study subjects

Table 1 depicts the demographic information: 111 subjects with normal cognition, 193 subjects with MCI, and 95 subjects with AD. There were no significant differences in age and education across the three groups. As expected, there was a significant difference in MMSE scores across the three groups ($P < .001$). The mean MMSE score in the patients with AD was 24 ± 2 (mean ± SD), consistent with mild AD dementia (Table 1). In addition, the global clinical dementia rating scores (CDR) among the three groups were compared. There were significant differences in global CDR scores across the three groups ($P < .001$). In agreement with the previous findings, >50% of the subjects with MCI and AD were *APOE* ε4 carriers [14]. There were significant differences in CSF Aβ42, *t*-tau, and *p*-tau across the three groups. Consistent with previous findings, the subjects with AD had the lowest CSF Aβ42 and the highest CSF *t*-tau and *p*-tau protein [15]. The demographic information on the subjects by gender is reported in the Supplementary data.

3.2. Significantly greater levels of CSF Ng in the APOE ε4 carriers

To study Ng in AD, CSF Ng levels were analyzed among the three groups: control, MCI, and AD. As shown in Fig. 1, the results showed that CSF Ng levels were significantly higher in the subjects with AD, followed by MCI, then normal controls (control vs. MCI, $P < .001$; controls vs. AD, $P < .0001$). The Y-axis of Fig. 1 expresses the mean of CSF Ng levels with their standard deviations.

To examine the association of the *APOE* ε4 genotype with CSF Ng, CSF Ng levels were compared between the *APOE* ε4 carriers and *APOE* ε4 noncarriers in the three groups. In the normal control group, the *APOE* ε4 carriers had a tendency to have higher CSF Ng compared to *APOE* ε4 noncarriers, although there was no statistical significance ($P = .06$; Fig. 2A). In the MCI group, the *APOE* ε4 carriers had significantly higher levels of CSF Ng compared to *APOE* ε4 noncarriers ($P = .001$). In the AD group, there was no statistical difference in CSF Ng levels between the *APOE* ε4 carriers and *APOE* ε4 noncarriers ($P = .57$). The Y-axis of Fig. 2A expresses the mean CSF Ng levels adjusted by age, education, and gender with 95% confidence intervals.

To confirm the effect of *APOE* ε4 on CSF Ng, the gene dose-effect of *APOE* ε4 on CSF Ng was analyzed. Across the entire sample, the CSF Ng levels were increased in a gene dose-dependent manner (heterozygous *APOE* ε4 vs. homozygous *APOE* ε4). This result confirmed that *APOE* ε4 is directly associated with CSF Ng levels (Fig. 2B). The Y-axis of Fig. 2B expresses the mean of CSF Ng levels with 95% confidence intervals.

We further explored the *APOE*-by-sex interaction on CSF Ng levels with a general linear model. Across the entire sample, the *APOE*-by-sex interaction on Ng levels was significant after adjusting for education, age, and diagnosis ($P = .007$). Female *APOE* ε4 carriers had significantly higher levels of CSF Ng compared to female noncarriers, whereas male carriers showed modestly higher levels of CSF Ng compared to male noncarriers (difference between means, 218; 2 in females, $P < .001$; 35.9 in males, $P = .401$).

3.3. Correlation of Ng with MMSE score, Aβ42, t-tau, and p-tau

To understand the mechanism underlying elevated Ng levels in *APOE* ε4 carriers, the correlations between CSF Ng and different variables were analyzed in this cohort using Pearson correlation test (Table 2). An inverse correlation between CSF Ng and MMSE scores was found ($r = -0.16$, $P = .001$). In addition, an inverse relationship between CSF Ng and Aβ42 levels was observed ($r = -0.34$, $P < .0001$). We further analyzed the relationship between Ng and tau protein. Strong positive correlations between Ng and *t*-tau protein, and also *p*-tau protein were observed ($r = 0.71$, $P < .0001$; $r = 0.67$, $P < .0001$, respectively).

3.4. Association of Ng with APOE ε4

To explore the relationship between *APOE* ε4 and CSF Ng, the potential association of CSF Ng with *APOE* genotype was analyzed using a general linear regression model, both with and without adjustment of other factors (Table 3). CSF Ng was significantly associated with *APOE* ε4 (standardized $\beta = 0.22$ (0.05); $P < .0001$) without controlling for other factors (model 1). A significant association of CSF Ng with *APOE* ε4 was found after adjusting for age, education, and gender ($\beta = 0.21$ (0.05); $P < .0001$; model 2). We further confirmed that Ng is significantly associated with *APOE* ε4 after adjusting for age, education, gender, and diagnosis ($\beta = 0.15$ (0.05); $P = .0029$; model 3). Finally, we examined the potential association of Ng with two typically assessed biomarkers of AD: CSF Aβ42 and tau. We found that the association of CSF Ng with *APOE* ε4 was no longer present after controlling for CSF amyloid and tau protein ($\beta = 0.01$ (0.04); $P = .84$; model 4).

4. Discussion

In this study, CSF neurogranin (Ng) levels were significantly greater in *APOE* ε4 carriers compared to *APOE* ε4 noncarriers with MCI. Comparison of CSF Ng levels in *APOE* ε4 carriers versus *APOE* ε4 noncarriers with normal cognition showed a similar trend. CSF Ng levels were increased in an *APOE* ε4 gene dose-dependent manner. In contrast, there was no difference in CSF Ng levels between *APOE* ε4 carriers and noncarriers with AD. CSF Ng levels were correlated with MMSE score, CSF *t*-tau, *p*-tau, and Aβ42 levels. CSF Ng levels were significantly associated with *APOE* ε4 independent of age, education, and gender.

Although *APOE* ε4 has been reported as a strong risk factor for AD for decades, the mechanism underlying the pathogenesis of AD in *APOE* ε4 carriers remains unclear. Our findings provide evidence that *APOE* ε4 carriers may undergo synaptic damage to confer risk of AD. To our knowledge, this is the first report of significantly increased CSF Ng in *APOE* ε4 carriers compared to noncarriers with MCI. Higher CSF Ng levels were also observed among control *APOE* ε4 carriers compared to noncarriers, although there was no statistical significance between the two groups. Given the modest number of cognitively normal participants who were *APOE* ε4-positive, a larger number may have yielded statistically significant findings. Regarding the subjects with AD, there was no significant difference in CSF Ng levels between *APOE* ε4 carriers and noncarriers. Collectively, these data suggest that significantly increased CSF Ng in *APOE* ε4 carriers with MCI reflects an early event in the pathogenesis of AD. This finding is consistent with previous studies, which showed that high CSF Ng levels in subjects with MCI-predicted progression to dementia [9,10].

In this study, the effect of *APOE* ε4 on Ng was shown to be gene dose-dependent. This result is congruent with the previous findings of Reiman et al., who reported that *APOE* ε4 gene dose was correlated with lower regional cerebral metabolic rate of glucose [16].

With regard to gender, we observed that the *APOE* ε4 effect on CSF Ng levels was significantly stronger in females than males. This result is consistent with Sampedro's findings that cerebral hypometabolism and atrophy were greater in female *APOE* ε4 carriers than male carriers [17]. These data suggest that increased CSF Ng levels, together with other reported metabolic and structural alterations in female *APOE* ε4 carriers, may contribute to a higher risk of AD in women [14,18,19].

Regarding the biology of Ng, it is localized in neuronal cell bodies and dendrites of cerebral cortex, and hippocampus [20]. Ng is implicated in long-term potentiation and visual-spatial learning [21,22]. Davidsson and Blennow found that the expression of Ng was reduced in AD brains [23]. In AD neocortical tissue, Ng mRNA translocation to dendrites was reduced, whereas in frontotemporal dementia, Ng mRNA translocation to dendrites was preserved [24,25]. Taken together, these findings suggest that Ng may play an integral role in the cascade of neural events leading to AD. Regarding the nature of CSF Ng, Kvartsberg's group reported that CSF Ng was the C-terminally truncated species [9]. It is speculated that enzymatic activities may generate C-terminal fragments. It is possible that multiple synaptic injuries may upregulate enzymatic activities to generate C-terminal fragments of Ng, which are released into the lymphatic system in close proximity to the synaptically injured dendritic trees of injured cells. Another explanation is that dying neurons may contribute to the elevated Ng level in *APOE* carriers with MCI.

Regarding *APOE* genotype and synaptic function, animal studies have shown that *APOE* isoforms encoded by different *APOE* alleles differentially regulate synaptic plasticity and repair [3]. White et al. demonstrated that after entorhinal cortex lesioning, compensatory sprouting in association with synaptophysin and GAP-43 was impaired in transgenic mice expressing human *APOE*4 compared with *APOE*3 transgenic mice [26]. Their results are consistent with the association of impaired synaptic plasticity with *APOE* ε4 genotype.

APOE ε4-targeted replacement mice showed reduced excitatory synaptic transmission and dendritic arborization, compared to *APOE* ε3-targeted replacement mice [27]. This finding indicates that *APOE* ε4 genotype may modulate postsynaptic function. Although mounting evidence shows an association of *APOE* genotype with synaptic function in animal models, the data regarding the *APOE* effect on synaptic function in human are limited. Our study provides *in vivo* evidence that the *APOE* isoforms may differentially regulate synaptic function in patients.

Although the correlations presented above do not imply any type of causation, a number of new investigative directions are suggested. In our study, the strongest correlation was found between Ng and tau protein. Yet, the pathway between synaptic injury and diffuse neuronal degeneration in AD remains to be determined, as tau pathology is well known to contribute to synapse degeneration and resulting dementia [28,29]. Although tau protein is considered to be primarily an axonal protein, recent studies show that tau is present in postsynaptic terminals of nondemented patients and AD patients, as well as in the somatodendritic compartment of cultured primary hippocampal neurons which were treated by Aβ oligomers [30,31]. Increased tau in CSF may be attributed to either a presynaptic-postsynaptic dysfunction of significant magnitude to be detectable, or that there is a primary postsynaptic injury. Aberrant function of tau and Ng protein mediated by *APOE* ε4 may represent a critical event in the pathogenesis of AD.

In this study, we note a second correlation between CSF Ng and Aβ42. This observation warrants further investigation to determine the extent to which amyloid pathology is associated with synaptic injury in *APOE* ε4 carriers. Emerging evidence suggests that Aβ-induced synaptic dysfunction is dependent on the elevation of cytoplasmic Ca^{2+} and N-methyl-D-aspartate (NMDA) receptor-mediated activity, and this process results in dendritic spine loss [32–34]. A recent study showed that Ng rescues Aβ-mediated depression in synaptic transmission in organotypic hippocampal slices [35]. Because Ng is localized in dendritic spines and involved in calcium-mediated NMDA receptor activity, the association of Ng with amyloid-induced pathology should be further investigated. Another possible explanation is that a correlation between CSF Ng and Aβ42 merely reflects the association of Aβ42 and tau. Indeed, when we controlled for CSF tau levels, the association between amyloid and Ng was no longer present. Of note, Kvartsberg et al. did not find a relationship between Aβ42 and Ng [9]. This discrepancy might be attributable to differences in the level of cognitive impairment as measured by MMSE between the two populations. Another interpretation of the lack of reproducibility compared to the referenced study may be due to the sample processing methodology and variability of polyclonal antibodies among lots used in the assay.

In summary, we found that CSF Ng levels were significantly elevated in *APOE* ε4 carriers with MCI compared to noncarriers. This finding indicates that postsynaptic injury may be an early pathologic event in *APOE* ε4 carriers who develop AD. CSF Ng levels were correlated with MMSE score, CSF tau, and Aβ42 levels. CSF Ng levels were likely associated with *APOE* ε4 and clinical diagnosis and independent of age, gender, and education. An association of Ng with *APOE* ε4 was not found after adjusting for CSF amyloid and tau

protein. We believe that the interaction among Ng, tau protein, and Aβ42 may contribute to an important mechanism underlying synaptic damage in *APOE* ε4 carriers who develop AD.

Our study has limitations: (1) The cross-sectional design used in our study does not permit us to address the sequence of the events that may lead to cognitive impairment as a result of elevated Ng, decreased Aβ42, and increased tau protein in CSF. A longitudinal study, with multiple repeated measures, to monitor the levels of CSF Ng, Aβ42, and tau protein in *APOE* ε4 carriers is needed to provide such information pertaining to possible mechanisms underlying synaptic damage in the *APOE* ε4 carriers who develop AD. (2) Because the ADNI cohort is a selected convenience sample of volunteers, sample selection bias should be taken into consideration for interpretating the data. (3) Our study finding is based on ADNI samples. The result needs to be confirmed in other samples, ideally in a longitudinal population. (4) With a cross-sectional design, correlation and association analyses are performed. There may be other unknown additional mediating factors underlying observed correlations between Ng and other variables. Our finding is interesting, in that it demonstrates a key pathway which presages further in-depth investigation of *in vivo* synaptic function in AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Mahley RW, Huang Y. Apolipoprotein E sets the stage: response to injury triggers neuropathology. Neuron. 2012; 76:871–85. [PubMed: 23217737]
2. Holtzman DM, Herz J, Bu G. Apolipoprotein E and apolipoprotein E receptors: normal biology and roles in Alzheimer disease. Cold Spring Harb Perspect Med. 2012; 2:a006312. [PubMed: 22393530]

3. Liu CC, Kanekiyo T, Xu H, Bu G. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. Nat Rev Neurol. 2013; 9:106–18. [PubMed: 23296339]
4. Selkoe DJ. Alzheimer's disease is a synaptic failure. Science. 2002; 298:789–91. [PubMed: 12399581]
5. Gong Y, Lippa CF. Review: disruption of the postsynaptic density in Alzheimer's disease and other neurodegenerative dementias. Am J Alzheimers Dis Other Demen. 2010; 25:547–55. [PubMed: 20858652]
6. Harigaya Y, Shoji M, Shirao T, Hirai S. Disappearance of actin-binding protein, drebrin, from hippocampal synapses in Alzheimer's disease. J Neurosci Res. 1996; 43:87–92. [PubMed: 8838578]
7. Shim KS, Lubec G. Drebrin, a dendritic spine protein, is manifold decreased in brains of patients with Alzheimer's disease and Down syndrome. Neurosci Lett. 2002; 324:209–12. [PubMed: 12009525]
8. Diez-Guerra FJ. Neurogranin, a link between calcium/calmodulin and protein kinase C signaling in synaptic plasticity. IUBMB Life. 2010; 62:597–606. [PubMed: 20665622]
9. Kvartsberg H, Duits FH, Ingelsson M, Andreasen N, Ohrfelt A, Andersson K, et al. Cerebrospinal fluid levels of the synaptic protein neurogranin correlates with cognitive decline in prodromal Alzheimer's disease. Alzheimers Dement. 2015; 11:1180–90. [PubMed: 25533203]
10. Kester MI, Teunissen CE, Crimmins DL, Herries EM, Ladenson JH, Scheltens P, et al. Neurogranin as a Cerebrospinal Fluid Biomarker for Synaptic Loss in Symptomatic Alzheimer Disease. JAMA Neurol. 2015; 72:1275–80. [PubMed: 26366630]
11. Shaw LM, Vanderstichele H, Knapik-Czajka M, Clark CM, Aisen PS, Petersen RC, et al. Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. Ann Neurol. 2009; 65:403–13. [PubMed: 19296504]
12. Gabriel KR. A simple method of multiple comparisons of means. J Am Stat Assoc. 1978; 73:724–9.
13. Freund RJ, Littell RC, Spector PC. SAS System for Linear Models. 1986
14. Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. JAMA. 1997; 278:1349–56. [PubMed: 9343467]
15. Rosenmann H. CSF biomarkers for amyloid and tau pathology in Alzheimer's disease. J Mol Neurosci. 2012; 47:1–14. [PubMed: 22058061]
16. Reiman EM, Chen K, Alexander GE, Caselli RJ, Bandy D, Osborne D, et al. Correlations between apolipoprotein E epsilon4 gene dose and brain-imaging measurements of regional hypometabolism. Proc Natl Acad Sci U S A. 2005; 102:8299–302. [PubMed: 15932949]
17. Sampedro F, Vilaplana E, de Leon MJ, Alcolea D, Pegueroles J, Montal V, et al. APOE-by-sex interactions on brain structure and metabolism in healthy elderly controls. Oncotarget. 2015; 6:26663–74. [PubMed: 26397226]
18. Payami H, Montee KR, Kaye JA, Bird TD, Yu CE, Wijsman EM, et al. Alzheimer's disease, apolipoprotein E4, and gender. JAMA. 1994; 271:1316–7. [PubMed: 8158809]
19. Poirier J, Davignon J, Bouthillier D, Kogan S, Bertrand P, Gauthier S. Apolipoprotein E polymorphism and Alzheimer's disease. Lancet. 1993; 342:697–9. [PubMed: 8103819]
20. Gerendasy DD, Sutcliffe JG. RC3/neurogranin, a postsynaptic calpacitin for setting the response threshold to calcium influxes. Mol Neurobiol. 1997; 15:131–63. [PubMed: 9396008]
21. Pak JH, Huang FL, Li J, Balschun D, Reymann KG, Chiang C, et al. Involvement of neurogranin in the modulation of calcium/ calmodulin-dependent protein kinase II, synaptic plasticity, and spatial learning: A study with knockout mice. Proc Natl Acad Sci U S A. 2000; 97:11232–7. [PubMed: 11016969]
22. Miyakawa T, Yared E, Pak JH, Huang FL, Huang KP, Crawley JN. Neurogranin null mutant mice display performance deficits on spatial learning tasks with anxiety related components. Hippocampus. 2001; 11:763–75. [PubMed: 11811671]
23. Davidsson P, Blennow K. Neurochemical dissection of synaptic pathology in Alzheimer's disease. Int Psychogeriatr. 1998; 10:11–23. [PubMed: 9629521]

24. Chang JW, Schumacher E, Coulter PM 2nd, Vinters HV, Watson JB. Dendritic translocation of RC3/neurogranin mRNA in normal aging, Alzheimer disease and fronto-temporal dementia. J Neuropathol Exp Neurol. 1997; 56:1105–18. [PubMed: 9329454]
25. Landry CF, Watson JB, Kashima T, Campagnoni AT. Cellular influences on RNA sorting in neurons and glia: an in situ hybridization histochemical study. Brain Res Mol Brain Res. 1994; 27:1–11. [PubMed: 7877439]
26. White F, Nicoll JA, Roses AD, Horsburgh K. Impaired neuronal plasticity in transgenic mice expressing human apolipoprotein E4 compared to E3 in a model of entorhinal cortex lesion. Neurobiol Dis. 2001; 8:611–25. [PubMed: 11493026]
27. Wang C, Wilson WA, Moore SD, Mace BE, Maeda N, Schmechel DE, et al. Human apoE4-targeted replacement mice display synaptic deficits in the absence of neuropathology. Neurobiol Dis. 2005; 18:390–8. [PubMed: 15686968]
28. Spires-Jones TL, Hyman BT. The intersection of amyloid beta and tau at synapses in Alzheimer's disease. Neuron. 2014; 82:756–71. [PubMed: 24853936]
29. Hyman BT. Amyloid-dependent and amyloid-independent stages of Alzheimer disease. Arch Neurol. 2011; 68:1062–4. [PubMed: 21482918]
30. Tai HC, Serrano-Pozo A, Hashimoto T, Frosch MP, Spires-Jones TL, Hyman BT. The synaptic accumulation of hyperphosphorylated tau oligomers in Alzheimer disease is associated with dysfunction of the ubiquitin-proteasome system. Am J Pathol. 2012; 181:1426–35. [PubMed: 22867711]
31. Zempel H, Thies E, Mandelkow E, Mandelkow EM. Abeta oligomers cause localized Ca(21) elevation, missorting of endogenous tau into dendrites, Tau phosphorylation, and destruction of microtubules and spines. J Neurosci. 2010; 30:11938–50. [PubMed: 20826658]
32. Palop JJ, Mucke L. Amyloid-beta-induced neuronal dysfunction in Alzheimer's disease: from synapses toward neural networks. Nat Neurosci. 2010; 13:812–8. [PubMed: 20581818]
33. Selkoe DJ. Soluble oligomers of the amyloid beta-protein impair synaptic plasticity and behavior. Behav Brain Res. 2008; 192:106–13. [PubMed: 18359102]
34. Tu S, Okamoto S, Lipton SA, Xu H. Oligomeric Abeta-induced synaptic dysfunction in Alzheimer's disease. Mol Neurodegener. 2014; 9:48. [PubMed: 25394486]
35. Kaleka KS, Gerges NZ. Neurogranin restores amyloid beta-mediated synaptic transmission and long-term potentiation deficits. Exp Neurol. 2015; 277:115–23. [PubMed: 26721336]

RESEARCH IN CONTEXT

1. Systematic review: The authors reviewed the literature using PubMed and AAIC meeting abstracts. CSF neurogranin (Ng) was significantly elevated in the subjects with Alzheimer's disease (AD) and mild cognitive impairment (MCI). The effect of *APOE* ε4 on Ng has not been reported. Relevant citations are appropriately cited.
2. Interpretation: Significantly higher CSF Ng levels in *APOE* ε4 carriers with MCI may reflect synaptic injury underlying early cognitive impairment. Neurogranin may be an early biomarker of AD and important for disease diagnosis and timing of intervention in *APOE* ε4 carriers.
3. Future directions: The study suggests that *APOE* ε4 carriers may undergo synaptic damage conferring risk of Alzheimer's disease. A longitudinal study should be designed to compare cognitive change between normal *APOE* ε4 carriers with high Ng and low Ng. A clinical outcome of the elevated Ng in *APOE* ε4 carriers should be investigated including brain structure and medication response.

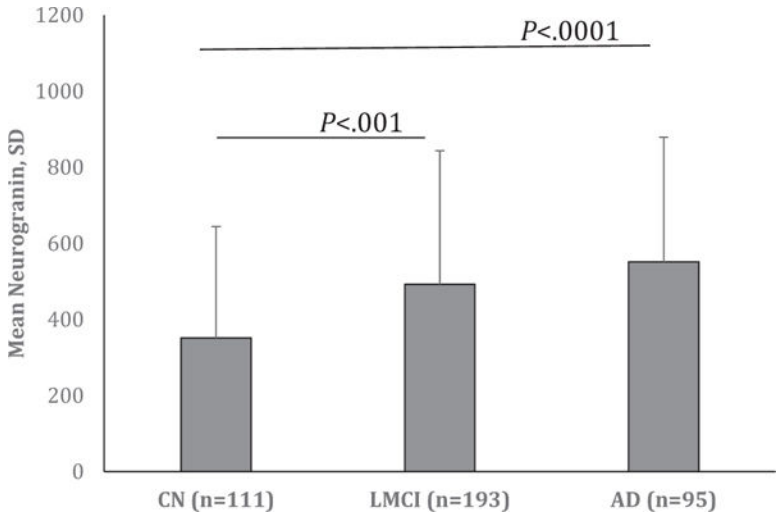

Fig. 1.

Significantly higher CSF neurogranin levels in the patients with MCI and AD compared to normal controls. Fig. 1 shows that CSF neurogranin levels are significantly higher in the subjects with MCI ($P < .001$) and AD ($P < .0001$) compared to that in subjects with normal cognition. The mean levels of CSF neurogranin with standard deviation are expressed on the Y-axis. Abbreviations: CSF, cerebrospinal fluid; MCI, mild cognitive impairment; AD, Alzheimer's disease. NOTE. The error bars represent standard deviation.

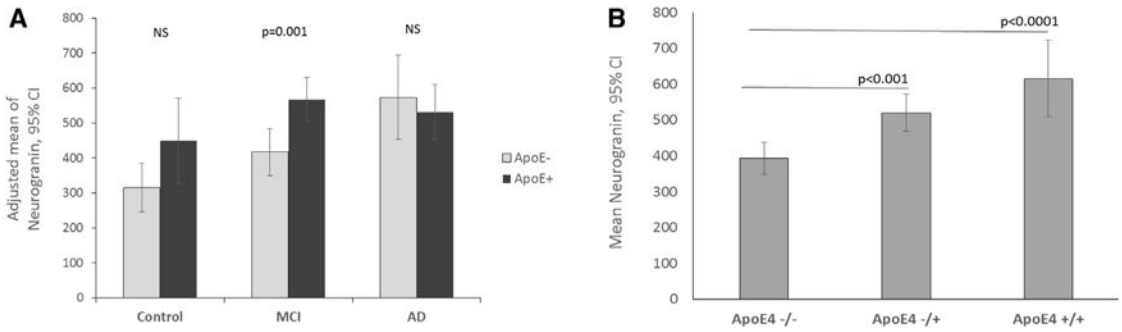

Fig. 2.

Comparison of CSF neurogranin levels in *APOE* ε4 carriers and *APOE* ε4 noncarriers with normal cognition, MCI and AD. (A) CSF neurogranin levels are significantly higher in *APOE* ε4 carriers with MCI compared to *APOE* ε4 noncarriers with MCI (P =.001). The Y-axis expresses the mean of CSF Ng levels adjusted for age, education, and gender with 95% confidence intervals. (B) The CSF neurogranin levels are significantly increased in a gene dose-dependent manner of *APOE* ε4 across the entire sample. The Y-axis expresses the mean of CSF Ng levels with 95% confidence intervals. Abbreviations: MCI, mild cognitive impairment; AD, Alzheimer's disease. NOTE. The error bars represent 95% confidence intervals.

Table 1

Demographic information on normal cognition, MCI and AD subjects

	Control (N = 111)	MCI (N = 193)	AD (N = 95)	*P* value
Age (y)	76 ± 5	74 ± 8	75 ± 8	.317
Education (y)	15.8 ± 2.8	15.7 ± 3.0	15.1 ± 3.2	.221
Female (%)	50[a]	33[b]	44	.01
APOE ε4 (%)	24[a]	53[b]	71[c]	<.001
MMSE	29[a] ± 1.0	27[b] ± 2.0	24[c] ± 2	<.001
CDGLOB	0[a] ± 0	0.5[b] ± 0	0.7[c] ± 0.3	<.001
Aβ42 (pg/mL)	206.0[a] ± 55.4	165.6[b] ± 54.5	143.2[c] ± 39.7	<.001
t-tau (pg/mL)	69.1[a] ± 29.90	102.2[b] ± 60.2	122.9[c] ± 57.4	<.001
p-tau (pg/mL)	24.9[a] ± 14.8	35.5[b] ± 18.1	41.4[c] ± 19.7	<.001

Abbreviations: MCI, mild cognitive impairment; AD, Alzheimer's disease; MMSE, mini-mental state examination; CDGLOB, global clinical dementia rating; *t*-tau, total tau protein; *p*-tau, phospho-tau protein; Aβ42, β-amyloid 42.

NOTE. Values are expressed as mean ± standard deviation. Alphabetic "a", "b", and "c" superscripts indicate that the pairwise groups have statistical significance with the Tukey HSD procedure after the Tukey-Kramer adjustment for multiple comparison of unequal sample sizes [12].

Table 2

Correlation between CSF Ng and MMSE, *t*-tau, *p*-tau, and Aβ42

	***t*-tau**	**Aβ42**	***p*-tau**	**MMSE**
CSF Ng				
R	0.71	−0.34	0.67	−0.16
P	<.0001	<.0001	<.0001	0.001

Abbreviations: CSF Ng, cerebrospinal fluid neurogranin; *t*-tau, total tau protein; Aβ42, β-amyloid 42; *p*-tau, phospho-tau protein; MMSE, mini-mental state.

NOTE. Pearson's correlation test was applied to analyze the correlation between CSF Ng and other variables. The correlation coefficient is expressed as *r*.

Table 3

Modeling of potential association of neurogranin with *APOE* ε4 adjusted for age, education, gender, clinical diagnosis, CSF tau, and Aβ 42

	Model							
	1		2		3		4	
	Beta (se)	***P***	**Beta (se)**	***P***	**Beta (se)**	***P***	**Beta (se)**	***P***
APOE ε4 (+) vs. (−)	0.22 (0.05)	<.0001	0.21 (0.05)	<.0001	0.15 (0.05)	.0029	0.01 (0.04)	.8405
Age, y			−0.06 (0.05)	.2268	−0.05 (0.05)	.2901	−0.09 (0.04)	.0152
Female vs. male			0.09 (0.05)	.0622	0.11 (0.05)	.0264	0.02 (0.04)	.6258
Education, y			−0.08 (0.05)	.1208	−0.07 (0.05)	.1545	−0.08 (0.04)	.0300
MCI vs. control					0.18 (0.06)	.0032	−0.03 (0.05)	.5614
AD vs. control					0.19 (0.06)	.0022	−0.08 (0.05)	.0751
t-tau							0.70 (0.04)	<.0001
Aβ42							−0.07 (0.04)	.1336

Abbreviations: MCI, mild cognitive impairment; AD, Alzheimer's disease; *t*-tau, total tau protein; CSF Ng, cerebrospinal fluid neurogranin; Aβ42, β-amyloid 42. Beta is standardized beta.

NOTE. General linear regression models were constructed to explore the association of neurogranin with other variables. Model 1 was unadjusted; model 2 was adjusted for age, sex, and education attainment; model 3 was additionally adjusted for diagnostic status; and model 4 was additionally adjusted for tau and Aβ42 levels. Beta is standardized beta.

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