APOE epsilon 4 carriers may undergo synaptic damage conferring risk of Alzheimer's disease

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ABSTRACT

Objective

Apolipoprotein E ε4 (*APOE* ε4) is a strong genetic risk factor of Alzheimer's disease (AD). The mechanism underlying AD pathogenesis in APOE ε4 carriers remains unclear. We

hypothesize that apoE isoforms (encoded by different *APOE* alleles) have differential effects on synaptic function.

Methods

A total of 399 Alzheimer Disease Neuroimaging Initiative (ADNI) subjects with normal cognition, mild cognitive impairment (MCI) and AD were studied. We compared the levels of CSF neurogranin (Ng), a postsynaptic maker, between APOE ε4 carriers and non-carriers. We examined associations of age, education, gender, and CSF amyloid beta 42 (Aβ42), total tau (t-tau), and phosphorylated tau (p-tau) with Ng levels.

Results

Neurogranin levels were significantly higher in APOE ε4 carriers with MCI, as compared to APOE ε4 non-carriers with MCI (?) (569.27±32.83 v.s. 403.03±37.23, p=0.01). There was no difference in the levels of Ng between the APOE ε4 carriers and APOE ε4 non-carriers with AD (541.34±37.02 v.s. 575.05±72.76, p=0.57). The levels of CSF Ng were correlated with MMSE (r=-0.16, p=0.001), levels of tau (r=0.76, p<0.0001), p-tau (r=0.61, p<0.0001) and amyloid 42 (r=-0.34, p<0.0001). The levels of CSF Ng were significantly associated with APOE ε4 independent of age, education and gender.

Interpretation

Significantly elevated CSF Ng levels in APOE ε4 carriers with MCI may reflect synaptic injury associated with early cognitive impairment. Associations of CSF Ng with Aβ42 and tau support the role of synaptic dysfunction in AD. Neurogranin may be an early clinical biomarker of AD for disease diagnosis and timing of intervention in APOE ε4 carriers.

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 the 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 (aMCI), a prodromal stage of AD, have lower beta β-amyloid 42 (Aβ42), elevated total tau (t-tau) and phospho-tau (p-tau) in cerebrospinal fluid (CSF), compared to APOE ε4 non-carriers[2]. Structural magnetic resonance imaging (MRI) studies, independent of CSF study, have shown that APOE ε4 carriers with AD and MCI have more severe brain atrophy in the parietal and temporal lobe and hippocampus, compared to APOE ε4 non-carriers[3]. Taken together, it is suggested that the APOE ε4 genotype has multiple effects on the brain metabolism and structure.

Currently, the exact mechanism by which the APOE ε4 genotype contributes to the early onset and rapid progression of the disease remains unclear. Although the APOE ε4 genotype affects amyloid metabolism, a lack of consistent association between amyloid deposition and cognitive impairment 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 the cognitive impairment in AD[4]. Additionally, neuropathological studies demonstrate that post-synaptic components are damaged in AD[5]. Neurogranin (Ng) is a post-synaptic protein, which is highly expressed in hippocampus and involved in memory consolidation[6]. Kvartsberg et al. report that Ng is markedly elevated in CSF of patients with AD and MCI, indicating that Ng can be a biological marker reflecting synaptic integrity[7]. We hypothesize that APOE ε4 has detrimental effects on synaptic function, leading to the elevated level of Ng, which may in turn contribute to the cognitive impairment in the APOE ε4 carriers of AD.

To investigate the effect of APOE ε4 on CSF Ng, CSF Ng levels were examined in subjects with normal cognition, MCI and AD from the ADNI dataset. We compared the

levels of CSF Ng between APOE ε4 carriers and APOE ε4 non-carriers among these subjects. We examined the gene dose effect of APOE ε4 on the levels of the CSF Ng. We examined the correlation between Ng and mini mental state examination (MMSE), CSF amyloid, tau protein, and phospho-tau protein. Finally, we analyzed the association of APOE ε4 with Ng by controlling for age, education, gender, clinical diagnosis and CSF Aβ42, t-tau and p-tau.

MATERIALS AND METHODS

ADNI Study

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (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). In our study, the subjects with an initial analysis of Ng were included. Institutional review board approval is obtained at each ADNI site, and informed consent is obtained from each participant or authorized representative. Demographic information is extracted from the ADNI database. In this study, there are 111 subjects with normal cognition, 193 subjects with MCI, and 95 subjects with AD.

CSF Analyses

Quantification of neurogranin (Ng) in CSF:

The levels of the CSF Ng were determined at the Clinical Neurochemistry Laboratory, University of Gothenburg, Sweden, results are available from ADNI database. As described previously in detail (REF=Kvartsberg), CSF Ng was analyzed by electrochemi-luminescence technology (Meso Scale Discovery, Gaithersburg, Maryland, USA) using Ng 7, which is a monocloncal antibody specific for Ng, as coating antibody and polyclonal Ng anti-rabbit (ab 23570, Upstate) as a detector antibody. Values are given as pg/mL.

Quantification of amyloid 42, total tau and phospho-tau in CSF:

The levels of amyloid 42, total tau (T-tau) and phosphorylated tau (P-tau) were analyzed 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 results are available from ADNI database (UPENN-Biomarker data [ADNI1]). As described previously (REF = Shaw et al, Ann Neurol 2009;65:403-413), the xMAP Luminex platform and Innogenetics/Fujirebio AlzBio3 immunoassay kits were used following the SOP in place at the UPenn/ADNI Biomarker Laboratory, according to the kit manufacturer's instructions Acceptance criteria as documented in the UPenn/ADNI Biomarker Laboratory SOP were followed for these analyses. Individual sample results were acceptable in all cases except where noted and those are reported as "NA" in the CSV file "BIOMARK5". Linear regression analyses (Passing-Bablock) were performed for Aβ1-42 and t-tau comparing CSF concentration results obtained.

APOE Alleles genotyping:

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

Statistical Analysis

The F test was used to examine the differences in continuous variables and the $\chi 2$ test was used to compare the frequencies of categorical variables among control, MCI and AD groups. Statistically significant results for individual variables were followed by post-hoc pairwise comparisons with the Tukey's HSD method. To examine the association between APOE ε4 genotype and Ng levels, a series of 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 cognition status; and model 4 was additionally adjusted for biomarker Tau and amyloid 42 levels. All data analyses were performed with SAS statistical software version 9.3 (SAS institute, Cary, NC) and the level of statistical significance was set at $p < 0.05$.

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RESULTS

Demographic information of study subjects:

Table 1 depicts the demographic information of the subjects. There were 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, ($P< 0.001$). The mean MMSE score in the patients with AD was 24 ± 2, consistent with mild AD dementia (Table 1). In agreement with the previous findings, over 50% of the subjects with MCI and AD were APOE ε4 carriers[8]. There was a significant difference in CSF Aβ42, t-tau, and p-tau across the three groups. Consistent with the previous findings, the subjects with AD had the lowest CSF amyloid 42, highest total tau protein and phospho-tau protein[9].

Significantly greater levels of the CSF Ng in the APOE ε4 carriers:

To study Ng in AD, the levels of CSF Ng were analyzed among the three groups: control, MCI, and AD. Results showed that the CSF Ng was significantly higher in the subjects with AD, followed by MCI, then normal control ($p<0.001$) (Figure 1). The results are consistent with the previous finding reported by Kvartsberg et al[7]. To examine the association of APOE ε4 genotype with the CSF Ng, the levels of CSF Ng were compared between APOE ε4 carriers and APOE ε4 non-carriers of the three groups. In the normal control group, APOE ε4 carriers had a tendency to have higher CSF Ng, compared to APOE ε4 non-carriers, although the difference only approached statistical significance ($p=0.06$) (Figure 2a). In the MCI group, APOE ε4 carriers had significantly higher levels of CSF Ng, compared to that of APOE ε4 non-carriers ($p=0.001$). In the AD group, there was no statistical difference in the levels of CSF Ng between APOE ε4 carriers and APOE ε4 non-carriers ($p=0.57$). To confirm the effect of APOE ε4 in CSF Ng, the gene dose effect of APOE ε4 in the levels of the CSF Ng was analyzed. Across the entire sample it was shown that the levels of CSF Ng were increased in a gene dose-dependent manner (heterozygous APOE ε4 v.s. homozygous APOE ε4), confirming that APOE ε4 is directly associated with the levels of Ng (Figure 2b).

Correlation of Ng with MMSE, amyloid 42, total tau, and phospho-tau:

To understand the mechanism underlying the elevated Ng in the APOE ε4 carriers, the correlation between Ng and global cognition was analyzed in this cohort (Table 2). An inverse correlation between Ng and MMSE scores was found (r= -0.16, p=0.001). In addition, an inverse relationship between Ng and amyloid 42 was observed. (r = -0.34, p<0.0001). We further analyzed the relationship between Ng and tau protein. The strong positive correlation between Ng and total-tau protein, and phosph-tau protein was observed (r=0.71, p< 0.0001; r=0.67, p< 0.0001 respectively).

Association of Ng with APOE ε4:

To further define the relationship between APOE ε4 and Ng, the association of Ng with APOE genotype, with or without controlling other factors, was analyzed using a generalized linear model (Table 3). Ng was significantly associated with APOE ε4 (standardized β = 0.22 (0.05); p<0.0001) without controlling other factors (Model 1). A significant association of Ng with APOE ε4 was found after adjusting for age, education, and gender (β = 0.21(0.05); p<0.0001) (Model 2). We further confirmed that Ng is significantly associated with APOE ε4 after adjusting for age, education, gender, and diagnosis (β = 0.15 (0.05); p=0.0029) (Model 3). Finally, we examined the association of Ng with AD CSF biomarkers. We found that the association of Ng with APOE ε4 was no longer present after controlling for CSF amyloid and tau protein (β = 0.01 (0.04); p=0.84) (Model 4).

DISCUSSION

In this study, the levels of CSF neurogranin (Ng) were significantly greater in APOE ε4 carriers with MCI, as compared to that of the APOE ε4 non-carriers, and a similar trend was found among controls. CSF Ng levels were increased in an *APOE* ε4 gene dose-dependent manner. In contrast, there was no difference in the levels of Ng between APOE ε4 carriers and non-carriers with AD dementia. CSF Ng levels were correlated with MMSE, t-tau, p-tau and Aβ42. 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 of AD for decades, the mechanism underlying pathogenesis of AD in APOE ε4 carriers remains unclear. Our findings provide clinical evidence that synaptic damage in the APOE ε4 carriers can be

detected at an early stage of AD. In the current study, 53 % of the MCI subjects and 71 % of the AD subjects were APOE ε4 carriers, highlighting the importance of the APOE ε4 genotype in AD. To our knowledge, this is the first report of increased CSF Ng among MCI APOE ε4 carriers compared with MCI APOE ε4 non-carriers.

Higher levels of Ng were also observed among control APOE ε4 carriers as compared to APOE ε4 non-carriers, with the differences approaching statistical significance. Given the modest number of cognitively normal participants who were APOE positive, a larger number of APOE ε4 positive patients would have likely yielded more statistical power and resulted in statistically significant findings given the observed effect size. The effect of APOE ε4 in Ng is also shown to be gene dose dependent. The finding supports the previous study by Reiman and colleagues who reported that APOE ε4 gene dose is correlated with lower regional CMRgl[10].

There was no significant difference in CSF Ng concentrations between APOE ε4 carriers and non-carriers with AD. These data suggest that increase in CSF Ng reflects an early event in the pathophysiological process of AD in APOE ε4 carriers and that synaptic dysfunction might be a mechanism contributing to the clinical phenotype already in pre-dementia stages of the disease. This finding is consistent with a previous study reported by Kvartsberg et al.[7]. In their MCI cohort, high CSF Ng levels were found to predict progression to dementia regardless of APOE genotype.

Neurogranin, a post-synaptic protein, is implicated in the formation of long-term potentiation of synaptic plasticity. Ng knockout mice show severe deficits in visual-spatial learning. , Davidsson and Blennow found that the expression of Ng, along other synaptic proteins, is reduced in AD brains[11]. Chang reported that Ng mRNA is selectively detected in the dendrite and soma of neurons in the cerebral cortex and hippocampus[12]. In AD neo-cortex tissue, Ng mRNA translocation to dendrites is reduced, while Ng mRNA translocation to dendrites is preserved in frontotemporal dementia[12, 13]. Taken together, it is suggested that Ng might play an integral role in the cascade of neural events leading to AD. Since Ng reflects synaptic function *in vivo*, a prospective study with repeated CSF samplings examining Ng over the disease course in APOE ε4 carriers is likely to greatly enhance our understanding of AD pathogenesis.

Regarding APOE genotype and synaptic function, animal studies show that apoE

isoforms encoded by different *APOE* alleles differentially regulate synaptic plasticity and repair[14]. White et al. demonstrated that compensatory sprouting in association of synaptophysin and GAP-43 after entorhinal cortex lesion is impaired in transgenic mice expressing human apoE4, compared with apoE3 transgenic mice[15]. This indicates that the APOE ε4 genotype is involved in poor synaptic plasticity. APOE ε4 targeted replacement mice show reduced excitatory synaptic transmission and dendritic arborization, compared to the mice with APOE ε3 targeted replacement mice[16]. It indicates that APOE ε4 genotype modulates post-synaptic function. Although mounting evidence shows the association of APOE genotype with synaptic function in animal models, the findings of APOE effect on synaptic function in human is limited. Our study provides clinical *in vivo* evidence that the apoE isoforms may differentially regulate synaptic function in human subjects.

In this study, we found that the levels of CSF Ng correlate with MMSE, CSF amyloid 42, and tau protein. The strongest correlation was found between Ng and total tau protein, indicating a close relationship between synaptic injury and diffuse neuronal degeneration in AD, as tau pathology contributes more to synapse degeneration and resultant dementia[17, 18]. Although tau protein is primarily considered to be an axonal protein, recent studies show tau presents in postsynaptic terminals of non-demented human controls as well as AD cases[19,20]. Additional studies show that tau is important for targeting fyn kinase to the post-synaptic density[21,22, 23]. Further support for the involvement of tau in post-synaptic dysfunction was gained from studies showing that accumulation of P301L mutant tau in dendritic spines of cultured neurons is associated with disrupted synaptic transmission and altered neurotransmitter receptor composition of the post-synaptic density [24-27]. Taken together, our data suggest that tau protein and Ng might play a collective role in the post-synaptic compartment. Aberrant function of tau and Ng protein induced by APOE ε4 may represent a critical event in the pathogenesis of AD.

In this study, we also found a correlation between the CSF Ng and amyloid 42. While this may suggest that amyloid pathology is involved in the process of synaptic injury in the subjects with APOE ε4 genotype, another explanation is that this association merely reflects the association of amyloid 42 and tau in the CSF. Indeed, when we controlled for levels of CSF tau, the association between amyloid and NG was no longer present. Relationship between amyloid and synaptic injury has been reported in several studies[28],[29].

Emerging evidence suggests that Aβ-induced synaptic dysfunction is dependent upon the elevation of cytoplasmic Ca2+ and NMDA receptor -mediated activity[29-31]. This process results in dendritic spine loss. As Ng is localized in dendritic spine and involved in calcium mediated NMDA receptor activity, the association of Ng with amyloid-induced pathology should be further investigated. Although Kvartsberg et al. did not find a relationship between amyloid 42 and Ng[7], this discrepancy might be attributable to differences in their study sample.

The cross sectional design utilized 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 amyloid 42, and increased tau protein in CSF. A longitudinal study to monitor the levels of the CSF Ng, amyloid 42, and tau protein in the APOE ε4 carrier is needed to provide much needed information pertaining to possible mechanisms underlying synaptic dysfunction in the APOE ε4 carriers of AD.

In summary, we report that the levels of CSF Ng are significantly elevated in the APOE ε4 carriers who have MCI. These data indicate that that post-synaptic injury is an early pathological event in the APOE ε4 carriers. The levels of CSF Ng are correlated with MMSE, the levels of tau, p-tau and amyloid 42. Independent of age, gender, and education, the levels of Ng are associated with APOE ε4 and clinical diagnosis. Association of Ng with APOE ε4 was no longer present after controlling for CSF amyloid and tau protein. We believe the interaction among Ng, tau protein, and amyloid 42 might be an important mechanism underlying synaptic damage in the APOE ε4 carriers. Our study provides clinical evidence that APOE isoforms may have differential effects on the CSF Ng and that the synaptic damage associated with the APOE ε4 genotype might contribute to the clinical phenotype of early onset and rapid progression of the disease in the APOE ε4 carriers. A limitation of this and all studies of association is that there may be other unknown mediating factors in early AD that may underlie observed correlations between Ng and other variables. There is no question that understanding the APOE ε4 effect in the pathogenesis of AD will significantly improve the diagnosis and treatment of AD.

Further study is warranted to investigate whether the elevated CSF Ng represents a specific marker for post-synaptic damage in AD. Nevertheless, a significant elevation of the CSF Ng in the early stage of AD in the APOE ε4 carriers provides a useful *in vivo* clinical

marker for diagnosis, timing of intervention, and monitor of AD in association with cognitive impairment.

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Authorship:

X.S. conceived the study, analyzed the data, interpretation of the data, and drafted the

manuscript. C.D. analyzed the data and designed the study. B.L. contributed to the design of the study, interpretation of the data, and editing of the manuscript. E.C. contributed to interpretation of the data and editing of the manuscript. D.L. contributed to the design of the study, interpretation of the data, and editing of the manuscript. K.B. and H.Z. contributed to measurement of CSF Ng, interpretation of the data and editing of the manuscript. C. B.W. contributed to the design of the study, interpretation of the data, and editing of the manuscript.

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Table and Figure legend

Table 1 shows the demographic information of normal cognition, MCI and AD subjects. MCI, mild cognitive impairment; AD, Alzheimer's disease

Table 2 shows the correlation between CSF neurogranin and MMSE, total tau, phospho-tau and amyloid β 42. CSFNG, CSF neurogranin

Table 3 shows the association of neurogranin with APOE ε4 adjusted for age, education, gender, clinical diagnosis, CSF tau and amyloid β 42.

Figure 1 shows CSF neurogranin levels in the subjects with normal cognition, MCI and AD. MCI, mild cognitive impairment; AD, Alzheimer's disease

Figure 2 a. showing comparison of the CSF neurogranin levels in the APOE ε4 carriers and APOE ε4 non-carriers with normal cognition, MCI and AD. b. showing the levels of CSF neurogranin are increased in a gene dose manner of APOE ε4. MCI, mild cognitive impairment; AD, Alzheimer's disease

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