Cognitively normal *APOE* ε4 carriers have specific elevation of CSF SNAP-25

Omar H. Butt [a], Justin M. Long [a,d,e], Rachel L. Henson [a,d], Elizabeth Herries [a,d], Courtney L. Sutphen [a,d], Anne M. Fagan [a,d,e], Carlos Cruchaga [c,d,e], Jack H. Ladenson [f], David M. Holtzman [a,d,e], John C. Morris [a,d,e,f], Beau M. Ances [a,b,d,e], Suzanne E. Schindler [a,d,*], for the Alzheimer's Disease Neuroimaging Initiative[**]

[a] *Department of Neurology, Washington University, Saint Louis, MO, USA*
[b] *Department of Radiology, Washington University, Saint Louis, MO, USA*
[c] *Department of Psychiatry, Washington University, Saint Louis, MO, USA*
[d] *Knight Alzheimer Disease Research Center, Washington University, St. Louis, MO, USA*
[e] *Hope Center for Neurological Disorders, Washington University, St. Louis, MO, USA*
[f] *Department of Pathology and Immunology, Washington University, Saint Louis, MO, USA*

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ABSTRACT

Cerebrospinal fluid (CSF) synaptosomal-associated protein 25 (SNAP-25) and neurogranin (Ng) are recently described biomarkers for pre- and postsynaptic integrity known to be elevated in symptomatic Alzheimer disease (AD). Their relationship with Apolipoprotein E (*APOE*) ε4 carrier status, the major genetic risk factor for AD, remains unclear. In this study, CSF SNAP-25 and Ng were compared in cognitively normal *APOE* ε4 carriers and noncarriers (n = 274, mean age 65 ± 9.0 years, 39% *APOE* ε4 carriers, 58% female). CSF SNAP-25, not CSF Ng, was specifically elevated in *APOE* ε4 carriers versus noncarriers (5.95 ± 1.72 pg/mL, 4.44 ± 1.40 pg/mL, $p < 0.0001$), even after adjusting for age, sex, years of education, and amyloid status ($p < 0.0001$). CSF total tau (t-tau), phosphorylated-tau-181 (ptau181), and neurofilament light chain (NfL) also did not vary by *APOE* ε4 status. Our findings suggest *APOE* ε4 carriers have amyloid-related and amyloid-independent presynaptic disruption as reflected by elevated CSF SNAP-25 levels. In contrast, postsynaptic disruption as reflected by elevations in CSF neurogranin is related to amyloid status.

1. Background

Apolipoprotein E (*APOE*) genotype is the major genetic risk factor for Alzheimer disease (AD) and is thought to modify both amyloid- (Fleisher et al., 2013; Kok et al., 2009; Liu et al., 2017; Morris et al., 2010), and tau-related pathology (Shi et al., 2017; Shi et al., 2019). *APOE* genotype has also been implicated in a variety of neurodegenerative disorders including α-synucleinopathies such as Parkinson disease and Lewy Body dementia (Li et al., 2004; Zhao et al., 2020), Huntington disease (Panegyres et al., 2006), frontotemporal dementia (Agosta et al., 2009), and chronic traumatic encephalopathy (McKee et al., 2009). The mechanism by which *APOE* genotype affects these diverse disorders remains unclear. However, multiple studies have highlighted amyloid-independent toxicity through synapse related pathways (Dumanis et al., 2009; Love et al., 2006; Nwabuisi-Heath et al.,

2014; Tannenberg et al., 2006; Wang et al., 2005; Zhao et al., 2020).

The *APOE* ε4 allele has been implicated in both presynaptic and postsynaptic dysfunction. This includes reductions of key presynaptic proteins (Tannenberg et al., 2006) and disruptions of presynaptic vesicular release and glutamine-to-glutamate production (Dumanis et al., 2013). Postsynaptic effects include disruptions of reelin-mediated long-term potentiation and plasticity (Weeber et al., 2002) and reductions in dendritic spine density and complexity (Dumanis et al., 2009; Jain et al., 2013; Wang et al., 2005) that may be further amplified in the presence of amyloid plaques (Holtzman et al., 2000). However, the relationship between *APOE* genotype and pre- or postsynaptic dysfunction in cognitively normal older adults remains unclear.

Two recent cerebrospinal fluid (CSF) biomarkers have emerged for assessing synaptic integrity in humans: synaptosomal-associated protein 25 (SNAP-25) and neurogranin (Ng). SNAP-25 is a component of the presynaptic SNARE complex, which is essential for vesicular trafficking (Shin, 2014). Ng is expressed in postsynaptic dendritic spines (Chang et al., 1997). Both CSF SNAP-25 (Brinkmalm et al., 2014; Zhang et al., 2018), and Ng concentrations (De Vos A, et al., 2015; Kester et al., 2015; Kvartsberg et al., 2015a; Kvartsberg et al., 2015b; Portelius et al., 2015; Tarawneh et al., 2016; Thorsell et al., 2010) are elevated in individuals with AD dementia.

In this study, we compared levels of CSF SNAP-25 and Ng in cognitively normal individuals as a function of *APOE* ε4 status. We adjusted for the effects of age, sex, years of education and amyloid status. Additionally, we evaluated levels of CSF total tau (t-tau), tau phosphorylated at position 181 (ptau181), and neurofilament light chain (NfL). Finally, we replicated our major finding in an independent cohort.

2. Materials and methods

2.1. Participants

The primary cohort consisted of participants enrolled at the Knight Alzheimer Disease Research Center (Knight ADRC) at Washington University in St Louis. Inclusion criteria were the following: participants who were cognitively normal (Clinical Dementia Rating [CDR] 0; Morris, 1993), had *APOE* genotype data, and had undergone analysis of CSF SNAP-25 and/or Ng. Methods for recruitment and assessment have previously been described (Morris et al., 2019). This study was approved by the Washington University Institutional Review Board and each participant provided signed informed consent.

2.2. Genetic analyses

DNA samples were collected at enrollment and genotyped using either an Illumina 610 or Omniexpress chip, as previously described (Cruchaga et al., 2013). *APOE* ε4 carriers were defined by the presence of at least one ε4 allele (ε2/ε4, ε3/ε4, or ε4/ε4) in contrast to *APOE* ε4 noncarriers (ε2/ε2, ε2/ε3, or ε3/ε3).

2.3. CSF acquisition and processing

Participants underwent CSF collection as previously described (Fagan et al., 2006). Briefly, CSF was collected at 8 AM after overnight fasting in a polypropylene tube via gravity drip using an atraumatic Sprotte 22 gauge spinal needle. Samples were gently inverted and centrifuged at low speed to pellet any cellular debris. CSF was then aliquoted into 500 μL volumes in polypropylene tubes and stored at -80°C until the time of assay.

CSF Aβ42, t-tau, and ptau181 were measured with corresponding Elecsys immunoassays on the Roche cobas e601 analyzer (Schindler et al., 2018). Amyloid status was established per previously published cutoffs for CSF ptau181/Aβ42 (Schindler et al., 2018), with individuals with a CSF ptau181/Aβ42 ratio $\leq$0.0198 categorized as amyloid-negative and individuals with ptau181/Aβ42$>$0.0198 categorized as amyloid-positive.

CSF SNAP-25 and Ng were measured via the microparticle-based immunoassay, Single Molecule Counting Erenna system (EMD Millipore, Burlington MA) system, with antibodies developed in the laboratory of Dr. Jack Ladenson at Washington University. CSF NfL was measured with an immunoassay kit manufactured by Uman Diagnostics (UmanDiagnostics, Umeå, Sweden).

2.4. PET image acquisition and processing

Amyloid positron emission tomography (PET) images were acquired on a subset of participants per previously described methods (Mintun et al., 2006; Su et al., 2015; Su et al., 2018; Su et al., 2019) using either [^{11}C] Pittsburgh Compound B (PiB) or florbetapir (^{18}F-AV-45). Standard uptake value ratios (SUVR) were calculated for the 30-60 minute postinjection window for PiB and 50-70 minutes for ^{18}F-AV-45. Raw PET data were then processed using a PET Unified Pipeline (github.com/ysu001/PUP). FreeSurfer 5.3 was employed for region of interest (ROI) segmentation. For each region, a tissue mask was generated based on segmentation, and partial volume correction performed (Su et al., 2015). SUVRs, also known as regional target-to-reference intensity ratios, were evaluated for each region using the cerebral cortex as the reference region. The partial volume corrected SUVR derived from cortical regions was used as a summary value for each PET imaging modality. To standardize across PiB and ^{18}F-AV-45, SUVRs were converted to centiloids (Klunk et al., 2004; Su et al., 2018).

2.5. Statistical analyses

Testing between subgroups was compared using unpaired t-tests for continuous variables and chi-square testing for categorical variables. Secondary validation for multiple comparisons was performed by calculating a false discovery rate. Analysis for covariance were implemented using the Matlab function LinearModel.fit between CSF SNAP-25 or Ng and *APOE* ε4 status, amyloid status, age, gender (female), and years of education.

2.6. Data availability policy

Data are available to qualified investigators upon request to the Knight ADRC (https://knightadrc.wustl.edu/Research/ResourceRequest.htm)

2.7. Replication cohort

For replication of the major finding, data were also 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), PET, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early AD. For up-to-date information, see www.adni-info.org. Inclusion criteria were identical to the primary cohort: participants who were cognitively normal (CDR 0), had available *APOE* genotype data, and had undergone analysis of CSF SNAP-25 and/or Ng using consistent assay lot number.

Table 1
Knight Alzheimer Disease Research Center (ADRC) participant characteristics

Group	All	Amyloid−	Amyloid+	p
N	274	191	83	
Age (years ± SD)	65 ± 9.0	62.3 ± 8.3	71.2 ± 7.4	< 0.0001
Education (years ± SD)	16.0 ± 2.50	16.1 ± 2.41	15.8 ± 2.70	N.S.
Sex (n, % Female)	159, 58%	116, 61%	43, 52%	N.S.
Race (n, %)				
Asian	1, <1%	1, <1%	0, 0%	N.S.
Black	23, 8%	19, 10%	4, 5%	N.S.
Non-Hispanic White	250, 91%	171, 90%	79, 95%	N.S.
APOE ε4 status[a] (n, %)	107, 39%	56, 29%	51, 61%	< 0.0001
PET Centiloid (mean ± SD)	13.3 ± 28.3	-2.14 ± 5.16	46.4 ± 29.4	< 0.0001
CSF Aβ_{42} (pg/mL)	1330 ± 620	1600 ± 540	730 ± 260	< 0.0001
CSF t-tau (pg/mL)	229 ± 106	188 ± 64.1	321 ± 124	< 0.0001
CSF ptau181 (pg/mL)	21.2 ± 11.8	16.5 ± 5.71	32.1 ± 14.6	< 0.0001
CSF SNAP-25 (pg/mL)	5.03 ± 1.72	4.66 ± 1.56	5.9 ± 1.76	< 0.0001
CSF Ng (pg/mL)	2080 ± 1120	1770 ± 880	2790 ± 1290	< 0.0001
CSF NfL (pg/mL)	1390 ± 690	1230 ± 620	1750 ± 690	< 0.0001
Group	*APOE* ε4−	*APOE* ε4+	p	
n	167	107		
Age (years ± SD)	64.9 ± 8.5	65.2 ± 9.78	N.S.	
Education (years ± SD)	16 ± 2.5	16 ± 2.58	N.S.	
Sex (n, % Female)	101, 60%	58, 54%	N.S.	
Race (n, %)				
Asian	1, 1%	0, 0%	N.S.	
Black	15, 9%	8, 7%	N.S.	
Non-Hispanic White	151, 90%	99, 93%	N.S.	
Amyloid status[a] (n, %)	32, 19%	51, 48%	< 0.0001	
PET Centiloid (mean ± SD)[b]	7.82 ± 26.0	22.9 ± 30.0	0.0004	
CSF Aβ_{42} (pg/mL)	1460 ± 630	1130 ± 550	< 0.0001	
CSF t-tau (pg/mL)	216 ± 100	249 ± 109	0.01	
CSF ptau181 (pg/mL)	19.8 ± 11	23.5 ± 12	0.01	
CSF SNAP-25 (pg/mL)	4.44 ± 1.4	5.95 ± 1.72	< 0.0001	
CSF Ng (pg/mL)	1970 ± 1070	2250 ± 1190	0.04	
CSF NfL (pg/mL)	1340 ± 680	1470 ± 690	N.S.	

Demographic comparison of amyloid-positive and amyloid-negative[a] groups, and *APOE* ε4 carriers and noncarriers. *P* values reflect between group comparisons using unpaired t-tests for continuous variables and chi-square tests for categorical variables. All participants are CDR 0.

[a] Amyloid-negative if CSF ptau181/Aβ_{42} <0.0198; Amyloid-positive if CSF ptau181/Aβ_{42} ≥0.0198 (Schindler et al., 2018).

[b] Average across the subset of participants who had PET Centiloid data; all participants underwent CSF testing.

3. Results

3.1. Participant characteristics

The Knight ADRC cohort consisted of 274 participants who met inclusion criteria. The characteristics of the cohort, grouped by either amyloid status based on CSF ptau181/Aβ42 or *APOE* ε4 carrier status, are shown in Table 1. There was no significant difference in years of education or gender by either amyloid status or *APOE* ε4 carrier status. Amyloid-positive individuals tended to be older and were more likely to carry an *APOE* ε4 allele ($p < 0.0001$). As expected, the individuals categorized as amyloid-positive by CSF ptau181/Aβ42 had significantly higher PET centiloid values. *APOE* ε4 carriers also had a higher average PET centiloid values ($p = 0.0004$).

3.2. Differences in CSF biomarkers by amyloid status or APOE ε4 carrier status

Concentrations of CSF biomarkers including Aβ42, t-tau, ptau181, SNAP-25, Ng, and NfL were examined as a function of amyloid status and or *APOE* ε4 carrier status. All six CSF biomarkers were significantly different between amyloid-positive individuals and amyloid-negative individuals ($p < 0.0001$). When grouped by *APOE* ε4 carrier status, significant group differences in Aβ42 ($p < 0.0001$), t-tau ($p = 0.01$), and ptau181 ($p = 0.01$) were observed (Table 1). Significant elevations in SNAP-25 ($p < 0.0001$) and Ng ($p = 0.04$) were also observed, although the difference in Ng did not survive after correction for multiple comparisons. Finally, no significant group difference in NfL was observed between *APOE* ε4 carriers and noncarriers.

### 3.3. CSF biomarkers in different APOE allele genotypes

CSF SNAP-25 and Ng as a function of *APOE* genotype were evaluated (Fig. 1). Presence of the *APOE* ε4 allele was associated with higher CSF SNAP-25 levels (Fig. 1A). In contrast, Ng levels did not vary consistently by *APOE* genotype (Fig. 1B). Similarly, no consistent relationship was observed between *APOE* ε4 carrier status and CSF t-tau, ptau181, or NfL (**Fig. S1**A-C).

3.4. CSF SNAP-25 and Ng by amyloid status

We next determined whether elevations in either CSF SNAP-25 or Ng were present when controlling for amyloid status in our cohort of cognitively normal participants (Fig. 2). Amyloid-positive individuals had higher CSF SNAP-25 levels ($p < 0.0001$; Fig. 2A). Among amyloid-negative individuals, *APOE* ε4 carriers had higher SNAP-25 levels ($p < 0.0001$); among amyloid-positive individuals, *APOE* ε4 carriers also had higher SNAP-25 levels ($p < 0.05$; Fig. 2C). In contrast, while amyloid-positive individuals had higher CSF Ng levels (Fig. 2B), there was no difference between *APOE* ε4 noncarriers or carriers after controlling for amyloid status (Fig. 2D).
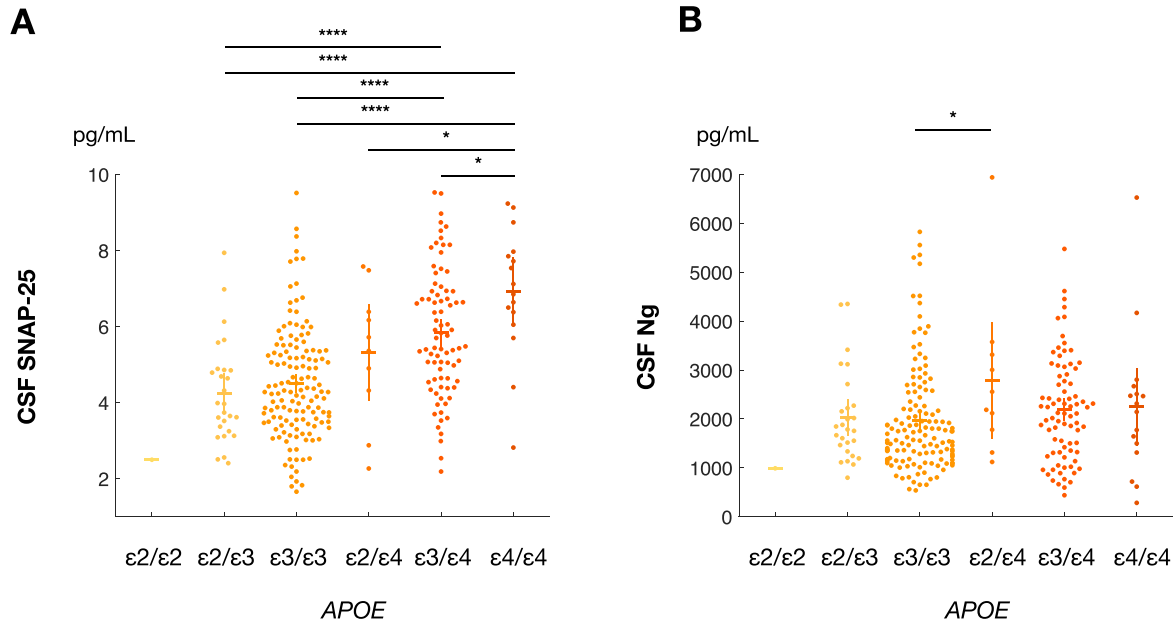
Fig. 1. CSF SNAP-25 and Ng by *APOE* Allele Genotype. Figure 1. Levels of CSF SNAP-25 (A) and Ng (B) by *APOE* genotype. CSF SNAP-25 was significantly elevated in *APOE ε*4 allele carriers compared to noncarriers. A similar relationship was *not* observed for CSF Ng; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$
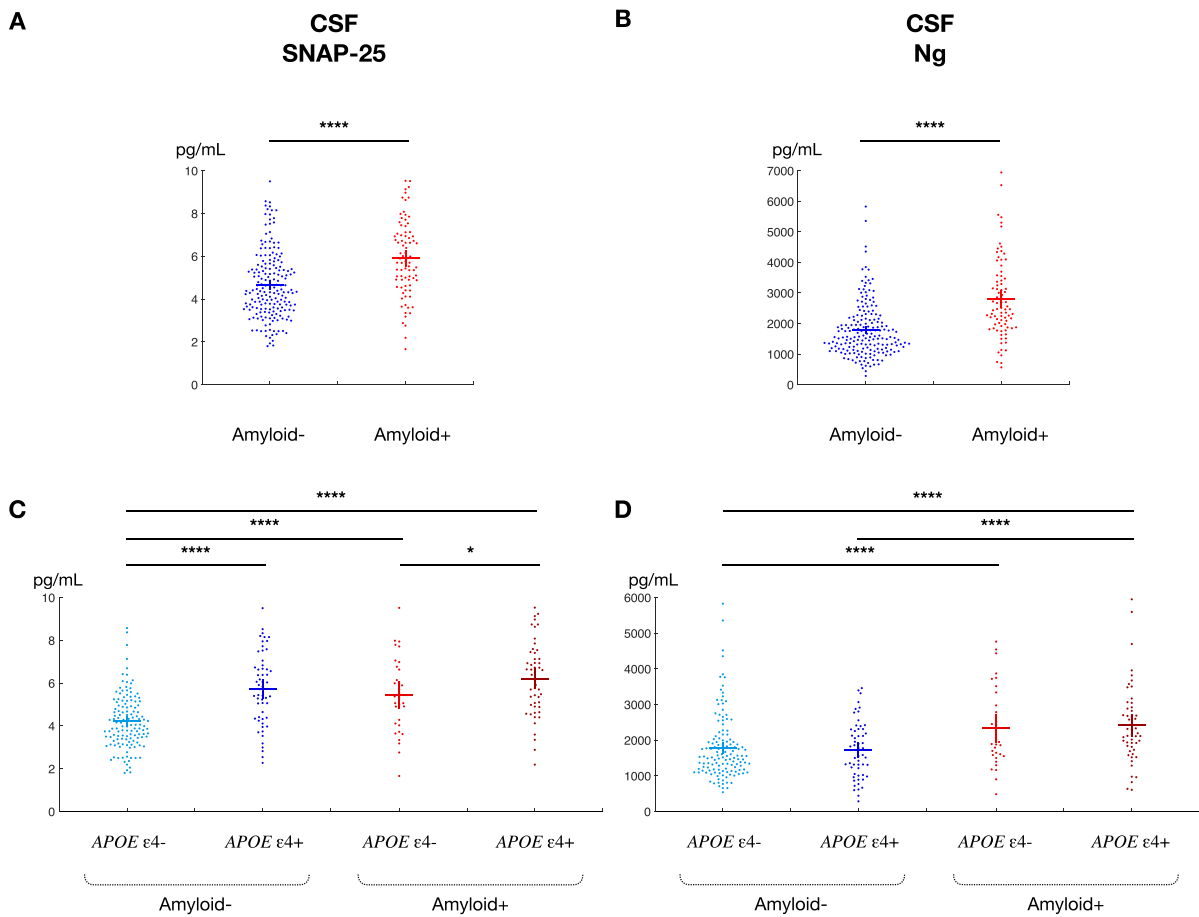
Fig. 2. CSF SNAP-25 and Ng by amyloid status. Figure 2. Unadjusted CSF SNAP-25 and Ng as a function of amyloid status and *APOE ε*4 carrier status with confidence intervals. CSF SNAP-25 (A) and Ng (B) are significantly higher in cognitively normal participants who are amyloid-positive compared to amyloid-negative[a]. Even after adjusting for amyloid status, CSF SNAP-25 (C) is greater in *APOE ε*4 carriers than *APOE ε*4 noncarriers. CSF Ng (D) does not vary by *APOE ε*4 carrier status. [a]Amyloid-negative if CSF ptau181/Aβ_{42} < 0.0198; Amyloid-positive if CSF ptau181/Aβ_{42} ≥ 0.0198 (36) *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

Table 2
Effects of amyloid status and *APOE* ε4 status on CSF SNAP-25 and Ng levels

Overall Model	SNAP-25				Ng			
	F-statistic		*p*		F-statistic		*p*	
	17.3		**< 0.0001**		**13.6**		**< 0.0001**	
Parameter	Estimate	Standard error	tStat	*p*	Estimate	Standard Error	tStat	*p*
APOE ε4 carrier	1.30	0.20	6.5	< 0.0001	55.70	133	0.42	N.S.
Amyloid positive	0.69	0.24	2.9	0.004	784	158	4.95	< 0.0001
Age (years)	0.012	0.012	0.98	0.33	23.9	7.87	3.04	0.003
Female sex	-0.24	0.19	-1.24	0.22	62.2	129	0.48	N.S.
Education (years)	-0.06	0.038	-1.58	0.12	-14.2	25.2	-0.56	N.S.
Intercept	4.89	1.14	4.3	< 0.0001	394	762	0.52	N.S.

CSF SNAP-25 and CSF Ng as a function of *APOE* ε4 carrier status and amyloid status[a], adjusting for age, sex, and years of education. The estimate reflects the pg/mL change in biomarker levels associated with a unit change in the predictor values.
[a]Amyloid-negative if CSF ptau181/Aβ_{42} < 0.0198; Amyloid-positive if CSF ptau181/Aβ_{42} ≥ 0.0198 (Schindler et al., 2018).

Table 3
Effects of Amyloid Status and *APOE* ε4 status on CSF SNAP-25 and Ng levels (ADNI)

Overall model	SNAP-25				Ng			
	F-statistic		*p*		F-statistic		*p*	
	2.39		**0.05**		1.93		0.105	
Parameter	Estimate	Standard Error	tStat	*p*	Estimate	Standard Error	tStat	*p*
APOE ε4 carrier	0.99	0.44	2.27	0.03	164.25	312.99	0.52	N.S.
Amyloid positive	0.17	0.36	0.48	0.64	423.9	258.14	1.64	N.S.
Age (years)	-0.01	0.03	-0.16	0.87	16.26	22.91	0.71	N.S.
Female Sex	0.68	0.38	1.78	0.08	437.14	272.9	1.6	N.S.
Education (years)	0	0.06	-0.07	0.94	7.61	41.88	0.18	N.S.
Intercept	3.61	2.76	1.31	0.2	23.35	1973.57	0.01	N.S.

CSF SNAP-25 and CSF Ng as a function of *APOE* ε4 carrier status and amyloid status[a], accounting for confounds of age, sex, and years of education for the ADNI dataset. The estimate reflects the pg/mL change in biomarker levels associated with a unit change in the predictor values.
[a]Amyloid-negative if CSF ptau181/Aβ_{42} <0.0198; Amyloid-positive if CSF ptau181/Aβ_{42} >0.0198.

3.5. Modeling CSF biomarker as function of amyloid status and APOE ε4 carrier status

All previous group comparisons were between unadjusted values for each CSF biomarker. Linear modeling was next used to examine the relationship between either CSF SNAP-25 or Ng and *APOE* ε4 carrier status, amyloid status, participant's age, sex, and years of education (Table 2). Linear modeling revealed that both *APOE* ε4 carrier status ($p < 0.0001$) and amyloid status ($p = 0.004$) were significant determinants of CSF SNAP-25 levels. In contrast, CSF Ng levels were affected by amyloid status ($p < 0.0001$) and age ($p = 0.003$), but not by *APOE* ε4 carrier status.

Identical models for CSF t-tau and ptau181 demonstrated only a clear relationship with amyloid status ($p < 0.0001$) and age ($p < 0.0001$) (**Table S1**). For CSF NfL, only age ($p < 0.0001$) and female sex ($p < 0.0001$) were significant determinants; no significant effect of amyloid status or *APOE* ε4 carrier status was observed (**Table S2**).

Finally, modeling was repeated using an independent cohort from the ADNI dataset (n = 57, mean age 76 ± 5.3 years, 21% *APOE* ε4 carriers, 40% female). In contrast to our Knight ADRC dataset, participants were older with a lower percentage of cognitively normal individuals who were *APOE* ε4 carriers and female participants (**Table S3**). The relationship between CSF SNAP-25, Ng, *APOE* ε4 carrier status, amyloid status, age, sex, and years of education was evaluated in the ADNI cohort using the same models as applied to the Knight ADRC cohort (Table 3). As before, CSF SNAP-25 levels were significantly higher in *APOE* ε4 carriers ($p = 0.03$). In this smaller cohort, CSF Ng levels were not significantly associated with any of the predictors. In summary, two independently collected datasets both reveal that CSF SNAP-25 levels are higher in cognitively normal *APOE* ε4 carriers, even after accounting for possible confounds.

4. Discussion

This study investigated CSF levels of the presynaptic marker SNAP-25 and the postsynaptic marker Ng in cognitively normal, older individuals. Presynaptic SNAP-25, but not postsynaptic Ng, was specifically elevated in the CSF of *APOE* ε4 carriers even after adjusting for age, sex, years of education, and amyloid status. The elevation of SNAP-25 but not Ng in *APOE* ε4 carriers may indicate selective presynaptic damage in *APOE* ε4 carriers; alternatively, Ng (or the Ng assay used in this study) may simply not be as sensitive to *APOE* ε4-related changes. CSF levels of t-tau, ptau181, and NfL also did not vary by *APOE* ε4 carrier status. These results are the first to demonstrate a relationship between CSF SNAP-25 elevation and *APOE* ε4 carrier status in cognitively normal older individuals without biomarker evidence of brain amyloidosis, and extend earlier reports of elevated CSF SNAP-25 levels in *APOE* ε4 carriers with early symptomatic AD (equivalent to mild cognitive impairment (MCI) due to AD and mild AD dementia (Galasko et al., 2019; Sutphen et al., 2018; Tible et al., 2020; Wang Q et al., 2018; Wang S et al., 2018; Zhang et al., 2018).

Previous work examining CSF SNAP-25 and Ng levels report that levels reach their maximum in individuals with early symptomatic AD, and then decline with progression to AD dementia (Sutphen et al., 2018). However, after accounting for *APOE* ε4 carrier status, differences between the cognitively normal and early symptomatic AD groups were present for Ng but not SNAP-25. This

suggests that differences in SNAP-25 were related to *APOE* ε4 carrier status rather than diagnosis. Wang S et al. (2018) also demonstrated significantly higher levels of CSF SNAP-25 in *APOE* ε4 carriers compared to noncarriers with MCI, but no significant relationship was observed for participants who were cognitively normal or who had dementia due to AD. Both studies relied on the ADNI dataset, which includes a sizeable number of participants with MCI or AD dementia, but comparatively fewer cognitively normal elderly participants. Cognitively normal *APOE* ε4 carriers are particularly under-represented in the ADNI cohort, but are well represented in the Knight ADRC cohort, explaining why our current findings were not previously observed. More recently, Galasko et al. (2019) reported elevations in CSF SNAP-25 and Ng in AD dementia compared to cognitively normal individuals, but did not specifically evaluate the effect of *APOE* genotype in cognitively normal individuals. Finally, Tible et al. (2020) also reported CSF SNAP-25 and Ng elevations in *APOE* ε4 carriers with AD and non-AD dementia, but again, the effects of *APOE* genotype in cognitively normal individuals were not evaluated.

A number of studies have demonstrated that *APOE* ε4 is associated with synaptic dysfunction. Neuropathologic analyses of human brain from normal *APOE* ε4 carriers demonstrate decreased protein levels of synaptic markers (Love et al., 2006). *APOE* ε4 targeted replacement (TR) mice exhibit progressive loss of dendritic arbors and lower levels of excitatory synaptic activity (Dumanis et al., 2009; Klein et al., 2010). *APOE* ε4 has been shown to interfere with endosome recycling and glutamate receptor function via effects on Reelin signaling (Chen et al., 2010). Isogenic iPSC-derived human neurons expressing *APOE* ε4 exhibit early synaptic maturation and reduced expression of a number of genes, most of which are associated with synaptic function (Lin YT et al., 2018). Thus, most of the previously described mechanisms have been restricted to the postsynaptic compartment.

The mechanism(s) underlying possible presynaptic dysfunction in cognitively normal *APOE* ε4 carriers remains unclear. In animal models, the *APOE* ε4 allele has been associated with decreased presynaptic protein levels in response to environmental factors (Levi et al., 2003). Subsequent studies using transgenic mice expressing human *APOE* (ApoE4-TR, ApoE3-TR, and ApoE2-TR) reveal disruptions in vesicular release of several neurotransmitters (Dolejší et al., 2016; Dumanis et al., 2013). ApoE4-TR mice demonstrated impaired glutaminase activity resulting in a net decrease in glutamate present in the nerve terminals not observed in ApoE2-TR or ApoE3-TR mice (Dumanis et al., 2013). More recent work reveals inhibition of hippocampal ACh release from cholinergic nerve terminals in ApoE4-TR mice in a choline acetyltransferase-independent manner (Dolejší et al., 2016). Human studies are more limited, with reports of decreased presynaptic protein levels in *APOE* ε4 carriers (Tannenberg et al., 2006).

There are several limitations of this study. It remains unclear whether *in vivo APOE* ε4 mediated disruptions in presynaptic glutamate or acetylcholine are associated with increases in interstitial or CSF SNAP-25 levels. Furthermore, the specific pathological change reflected by elevated CSF SNAP-25 levels in cognitively normal, amyloid-negative *APOE* ε4 carriers remains unclear. It is possible that changes in SNAP-25 levels in *APOE* ε4 carriers are not specific to the presynaptic compartment and instead reflect global synaptic dysfunction or loss that is not reflected in the levels of other CSF synaptic markers. This uncertainty further extends to the neuroanatomical localization for elevated SNAP-25 levels. It remains unclear whether elevations in SNAP-25 reflect a localized or more cortically distributed phenomena. Additional studies are needed to better characterize the source of CSF SNAP-25 and associated neuropathologic and neuroanatomic changes at the synaptic level in cognitively normal, amyloid-negative *APOE* ε4 carriers.

This study also duplicates a significant association of age and sex with CSF NfL levels (Khalil et al., 2020). No clear association between NfL and *APOE* ε4 or amyloid status was observed in our study, also as previously reported (Bos et al., 2019). Previous studies exploring NfL in healthy adults reported no change in association of CSF NfL with risk of early symptomatic AD after adjustment for *APOE* status (Kern et al., 2019). Further NfL elevations observed in dementia associated with Parkinson's disease (Lin YS et al., 2018) support NfL as a sensitive global marker of cumulative neural injury due to multiple etiologies rather than a highly specific marker of AD-related pathology.

5. Conclusions

Increased CSF SNAP-25 levels in cognitively normal *APOE* ε4 carriers, even those without brain amyloidosis, suggest that *APOE* ε4 may be associated with presynaptic dysfunction unrelated to amyloid. This difference is not seen with Ng, a postsynaptic marker, or another marker of neuronal injury, NfL. Differences in the longitudinal change of SNAP-25 in relation to *APOE* ε4 status remains unknown. It is also unclear if *APOE* ε4 carriers under 50 years old also have significant elevations in CSF SNAP-25. Further studies are needed to further discern the mechanism by which *APOE* ε4 modulates SNAP-25 levels and presynaptic dysfunction.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Author AMF is a member of the scientific advisory boards for Roche Diagnostics, Genentech and AbbVie and also consults for Araclon/Grifols, DiademRes, DiamiR and Otsuka Pharmaceuticals.

All remaining authors report no conflict of interest.

Credit author statement

Omar Butt: Conceptualization, Methodology, Software, Visualization, Formal Analysis, Writing-Original Draft, Validation **Justin Long:** Writing-Original Draft **Rachel Henson:** Investigation, Data Curation **Elizabeth Herries:** Investigation, Data Curation **Courtney Sutphen:** Investigation, Visualization **Anne Fagan:** Project administration, Resources, Funding acquisition **Carlos Cruchaga:** Resources **Jack Ladenson:** Resources **David Holtzman:** Writing-Original Draft **John Morris:** Resources, Funding acquisition **Beau Ances:** Conceptualization, Writing-Original Draft, Resources, Supervision **Suzanne Schindler:** Conceptualization, Methodology, Software, Formal analysis, Validation, Data Curation, Writing-Original Draft, Funding acquisition, Project Administration, Supervision.

Author contributions

O.H.B analyzed the data and wrote the manuscript. J.M.L aided in preparing the manuscript and provided critical manuscript review. R.L.H. ran samples and performed quality-control for assays. E.H. aided in the development, validation, and quality-control of the assays. C.L.S completed preliminary data analyses and ran samples. A.M.F provided critical manuscript review. C.C. provided critical manuscript review. D.M.H provided critical manuscript review. J.C.M. provided participant characterization and provided critical manuscript review. B.M.A. helped prepare the manuscript and provided critical manuscript review. S.E.S performed the initial data analysis, helped prepare the manuscript and provided critical manuscript review. All authors read and approved the final manuscript.

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Supplementary materials

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