

APOE4 Copy Number-Dependent Proteomic Changes in the Cerebrospinal Fluid¹

Miles Berger^{a,b,c,*}, Mary Cooter^a, Alexander S. Roesler^a, Stacey Chung^a, John Park^a, Jennifer L. Modliszewski^d, Keith W. VanDusen^a, J. Will Thompson^d, Arthur Moseley^d, Michael J. Devinney^a, Shayan Smani^{a,e}, Ashley Hall^a, Victor Cai^{a,e}, Jeffrey N. Browndyke^{b,c,f}, Michael W. Lutz^g, David L. Corcoran^d and Alzheimer's Disease Neuroimaging Initiative²

^aDepartment of Anesthesiology, Duke University Medical Center, Durham, NC, USA

^bCenter for Cognitive Neuroscience, Duke Institute for Brain Sciences, Durham, NC, USA

^cCenter for the Study of Aging and Human Development, Duke University Medical Center, Durham, NC, USA

^dDuke Center for Genomic and Computational Biology, Duke University, Durham, NC, USA

^eTrinity College of Arts and Sciences, Duke University, Durham, NC, USA

^fDepartment of Psychiatry & Behavioral Sciences, Duke University Medical Center, Durham, NC, USA

^gDepartment of Neurology, Duke University Medical Center, Durham, NC, USA

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Abstract.

Background: *APOE4* has been hypothesized to increase Alzheimer's disease risk by increasing neuroinflammation, though the specific neuroinflammatory pathways involved are unclear.

Objective: Characterize cerebrospinal fluid (CSF) proteomic changes related to *APOE4* copy number.

Methods: We analyzed targeted proteomic data from ADNI CSF samples using a linear regression model adjusting for age, sex, and *APOE4* copy number, and additional linear models also adjusting for AD clinical status or for CSF A β , tau, or p-tau levels. False discovery rate was used to correct for multiple comparisons correction.

Results: Increasing *APOE4* copy number was associated with a significant decrease in a CRP peptide level across all five models ($q < 0.05$ for each), and with significant increases in ALDOA, CH3L1 (YKL-40), and FABPH peptide levels ($q < 0.05$ for each) except when controlling for AD clinical status or neurodegeneration biomarkers (i.e., CSF tau or p-tau). In all models except the one controlling for CSF A β levels, though not statistically significant, there was a consistent inverse direction of association between *APOE4* copy number and the levels of all 24 peptides from all 8 different complement proteins measured. The odds of this happening by chance for 24 unrelated peptides would be less than 1 in 16 million.

Conclusion: Increasing *APOE4* copy number was associated with decreased CSF CRP levels across all models, and increased CSF ALDOA, CH3L1, and FABH levels when controlling for CSF A β levels. Increased *APOE4* copy number may also be associated with decreased CSF complement pathway protein levels, a hypothesis for investigation in future studies.

Keywords: Alzheimer disease, apolipoprotein E4, biomarker, C-reactive protein, cerebrospinal fluid, complement activation, mass spectrometry, neurogenic inflammation

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this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

*Correspondence to: Miles Berger, MD, PhD, Room 4317, Duke South Orange Zone, DUMC Box 3094, Durham, NC 27710, USA. Tel.: +1 919 684 8679; E-mail: miles.berger@duke.edu; Twitter: @RealMilesBerger

INTRODUCTION

The best described genetic contributor to late onset Alzheimer's disease (AD) is the $\epsilon 4$ polymorphism of the apolipoprotein E gene [1]. Individuals carrying a single *APOE4* allele copy have a ~ 3 -fold increased risk of developing AD, and those who carry two *APOE4* alleles have a greater than 10-fold risk of developing AD [2–5]. Additionally, the presence of an *APOE4* allele is associated with worse neurologic outcomes including a higher index of disability in multiple sclerosis patients, worse cognitive outcomes following mild traumatic brain injury, and increased risk of death following subarachnoid hemorrhage [6–8], as well as increased atherosclerotic cardiovascular disease risk [9]. Likely due to these pleiotropic effects, *APOE4* carriers live ~ 4.2 years less than non-*APOE4* carriers [10, 11]. Despite our knowledge of these multiple negative effects of *APOE4*, it remains unclear what the mechanisms are that explain how *APOE4* contributes to AD risk and worse outcomes across these other disease states.

The ApoE protein has multiple biological roles, including cholesterol transport in the central nervous system (CNS), signaling through cell surface receptors, and modulating synaptic function by regulating the expression of syntaxin-1, PSD95, and NMDA and AMPA receptors [12]. Given this multitude of functions, it is unclear which mechanisms explain the increased AD risk in *APOE4* carriers. Patients with an *APOE4* allele are typically diagnosed with AD in their 7th or 8th decade of life, even though the ApoE protein is expressed within the CNS throughout life [13]. This suggests that *APOE4* likely contributes to AD risk before cognitive deficits first appear [14, 15]. This idea is supported by fMRI studies demonstrating that young adult *APOE4* carriers without AD have significant alterations in the default mode network when compared to non-carrier controls [16]. Additionally, young adult *APOE4* carriers show increased activation of the bilateral medial temporal lobe during an encoding task [17], which may be a compensatory mechanism to achieve normal cognitive function in *APOE4* carriers.

One mechanism hypothesized to underlie the link between *APOE4* and AD is neuroinflammation. Indeed, neuroinflammation is a key contributor to AD pathogenesis in humans [18]. Recent evidence in murine models has shown that human *APOE4* knock-in mice have increased glial activation in response to intracerebroventricular LPS injection and increased IL-1 β , IL-6, and TNF α levels when

compared to *APOE2* or *APOE3* allele knock-in mice [19]. Furthermore, microglia isolated from *APOE4/4* targeted replacement mice, as compared to those from *APOE3/3* mice, have increased pro-inflammatory cytokines such as IL-6, TNF α , and IL12p40 [20, 21]. Although these studies have linked *APOE4* to increases in multiple inflammatory cytokines and pathways, it is unclear which of these inflammatory mechanisms are responsible for increased AD risk in *APOE4* carriers.

Because neurodegeneration in AD itself is associated with inflammation, it is important to study the effect of *APOE4* on the CNS of older adults who do not yet have dementia and neurodegeneration due to AD. Such studies provide an opportunity to discover how *APOE4* affects the CNS and how it increases AD risk, before frank AD-related neurodegeneration begins. Thus, here we analyzed targeted cerebrospinal fluid (CSF) proteomic data from Alzheimer's Disease Neuroimaging Institute (ADNI) research subjects, while controlling for AD clinical status, in order to find CSF protein level variation associated with *APOE4* allele copy number.

METHODS

ADNI study and participants

The patient data and clinical annotations used in this study were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (<http://adni.loni.usc.edu>). ADNI is a longitudinal multicenter study that tracks and evaluates changes in cognition, brain structure and function, and biomarkers associated with the progression of mild cognitive impairment (MCI) and AD [22]. Further detail on ADNI is found in the Acknowledgments section. Each ADNI site received written informed consent from all participants and institutional review board approval.

Inclusion and exclusion criteria for the normal control (NC), MCI, and AD cohorts is available at <http://adni.loni.usc.edu>. Briefly, NC subjects were defined as having a Mini-Mental State Examination (MMSE) [23] score ≥ 24 and Clinical Dementia Rating (CDR) [24] score of 0 and having no confounding neurological or psychological disorders. MCI subjects had MMSE scores of 23–30, a CDR score of 0.5, objective memory loss as measured by Wechsler Memory Scale Revised—Logical Memory II [25], and preserved activities of daily living. AD patients met the National Institute of Neurological

Table 1

Baseline characteristics of ADNI Patients, grouped by number of *APOE4* alleles. Values represent means (SD), or percentages in the case of gender (for females), or count per group (for AT classification)

| | 0 <i>APOE4</i> alleles (N = 148) | 1 <i>APOE4</i> allele (N = 104) | 2 <i>APOE4</i> alleles (N = 35) | p |
|--------------------------|----------------------------------|---------------------------------|---------------------------------|---------------------|
| Age | 75.94 (6.88) | 75.43 (6.77) | 71.86 (6.88) | 0.007 ¹ |
| Gender (Male) | 89 (60.1%) | 63 (60.6%) | 20 (57.1%) | 0.935 ² |
| Race | | | | 0.473 ³ |
| Asian | 3 (2.0%) | 0 (0.0%) | 0 (0.0%) | |
| Black/African American | 5 (3.4%) | 5 (4.8%) | 0 (0.0%) | |
| White | 140 (94.6%) | 99 (95.2%) | 35 (100.0%) | |
| Years of Education | 16 [14, 18] | 16 [14, 18] | 16 [14, 16] | 0.227 ⁴ |
| CSF A β * | 988.47 (397.74) | 643.16 (208.95) | 482.48 (160.98) | <0.001 ¹ |
| CSF Tau** | 273.08 (115.89) | 335.57 (109.98) | 348.32 (120.79) | <0.001 ¹ |
| CSF p-tau** | 25.72 (12.59) | 33.81 (12.73) | 35.64 (15.21) | <0.001 ¹ |
| Clinical Status | | | | <0.001 ² |
| Normal | 65 (43.9%) | 19 (18.3%) | 2 (5.7%) | |
| MCI | 64 (43.2%) | 53 (51.0%) | 18 (51.4%) | |
| AD | 19 (12.8%) | 32 (30.8%) | 15 (42.9%) | |
| (ATN) Classification**,+ | | | | <0.001 ³ |
| A-T- | 59 (40.4%) | 8 (7.8%) | 0 (0.0%) | |
| A+T- | 37 (25.3%) | 26 (25.5%) | 9 (25.7%) | |
| A-T+ | 13 (8.9%) | 3 (2.9%) | 0 (0.0%) | |
| A+T+ | 37 (25.3%) | 65 (63.7%) | 26 (74.3%) | |

p-value key: ¹ANOVA, ²Chi-square, ³Fisher's Exact, ⁴Kruskal Wallis. *28 patients not included who returned values >1700; 4 patients with no BL CSF measures. **4 patients with no BL CSF measures. +A+ defined as A β values below 1065 pg/ml, T+ defined as p-tau values over 27 pg/ml. N classification was not given, because it cannot be determined based on CSF biomarker levels.

and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRD) [26] criteria for probable AD and had MMSE scores of 20–26 and CDR scores of 0.5–1.0. The current study includes CSF samples from 289 unique ADNI-1 subjects (85 normal control, 134 MCI, and 66 AD patients). Publicly available metadata such as age, gender, diagnosis at baseline, MMSE score, and *APOE4* genotype were collected from the ADNI database. Cohort demographics are summarized in Table 1.

ADNI-1 CSF Collection and processing

CSF samples (0.5 mL) were obtained at ADNI visits, stored, transported, and processed according to published procedures [27, 28]. Technical details on the mass spectrometry platform data acquisition, quality control metrics, and validation protocols used in this study are described in the ADNI "Use of Targeted Multiplex Proteomic Strategies to Identify Novel CSF Biomarkers in AD" data primer and in [29]. Briefly, CSF samples were depleted of high abundance proteins using MARS-14 immunoaffinity resin, trypsin digested (1:10 protease:protein ratio), lyophilized, and desalted prior to LC/MRM-MS proteomic analysis on a QTRAP 5500 LC-MS/MS system. CSF multiplex multiple reaction monitoring (MRM) is a standardized peptide panel developed as a

QC metric to verify the reproducibility of sample processing and mass spectrometry analysis [30]. A total of 320 peptides produced by tryptic digestion of 143 proteins were identified and met the QC criteria of the ADNI working group for inclusion in the original dataset [29]. These peptides were selected to measure the levels of proteins previously implicated in AD neuropathology and/or neuro-inflammation [29].

Patients in the ADNI proteomics study were classified by AT (i.e., amyloid and p-tau) status, using previously reported CSF A β and p-tau measurements made with the Roche Elecsys platform, and previously described A β , and p-tau thresholds [31, 32]. We used the AT schema rather than the full ATN classification, because CSF tau and p-tau levels are highly co-linear, such that every patient who would be T+ would also be N+ and *vice versa* (Shaw LM, personal communication, 6/16/2020).

Statistical analysis

Mass spectrometry data from the ADNI study was re-analyzed to compare peptide data by *APOE4* allele count. Peptides with an expression value below zero were set to missing values. The intraclass correlation (ICC) across technical replicates was calculated for each peptide. Subsequent analysis included 294 peptides that had an ICC \geq 0.6. The technical replicate for each individual with the smallest number of

missing peptides was used in the analysis. We analyzed CSF targeted proteomic data from 289 research participants in the ADNI-1 study, 85 of whom were healthy controls, 134 of whom had MCI, and 66 of whom had dementia due to AD. Association between each of the variables of interest with each peptide was tested in a linear model framework with an empirical Bayes method for parameter estimation from the limma [33] Bioconductor [34] package. Age and gender were included as cofactors in all models; AD clinical status (i.e., normal, MCI, or dementia due to AD) was also included in model 2. Models 3, 4, and 5 also included either CSF tau or p-tau, or A β levels, respectively. False discovery rate was used to correct for multiple hypothesis testing within each statistical model.

RESULTS

ADNI patient cohort characteristics

Baseline characteristics of the ADNI-1 patients whose samples were used for targeted proteomics measurements [29] are presented in Table 1. Subjects with 0, 1, or 2 copies of the *APOE4* allele were similar in terms of gender, race, and years of education. Consistent with prior work showing that the *APOE4* allele is associated with reduced longevity [11], individuals with two *APOE4* allele copies were \sim 4 years younger those with zero or one copy of the *APOE4* allele. As expected, the percentage of patients with MCI and AD increased among patients with either 1 or 2 *APOE4* alleles. Consistent with prior work [35, 36], increasing *APOE4* copy number was associated with lower CSF A β levels. Increasing *APOE4* copy number was also associated with increases in CSF tau and p-tau levels (Table 1). Lastly, increasing *APOE4* copy number was associated with decreases in the proportion of patients who were A⁻T⁻ and increases in the proportion who were A + T⁺ (Table 1).

Model 1: CSF proteomic changes and *APOE4* gene dosage

To identify protein-derived peptides whose level(s) differed as a function of *APOE4* copy number, a linear model controlling for age and gender was used to test the relationship between *APOE4* copy number and CSF peptide levels. Initial analysis evaluated 294 peptides with sufficient replicability (ICC \geq 0.6) for measuring CSF expression variance by *APOE4* copy number (Table 2). In this model, 12

Table 2
Proteins included in the ADNI Targeted CSF Proteomics Study

| | | | | |
|-------|-------|-------|-------|-------|
| 1433Z | CMGA | IFNB | NELL2 | SCG3 |
| A1AT | CNDP1 | IGSF8 | NEO1 | SDCB1 |
| A1AT | CNTF | IL10 | NEUS | SE6L1 |
| A1BG | CNTN1 | IL12B | NFH | SHSA7 |
| A2GL | CNTN2 | IL17 | NFL | SIAE |
| A2MG | CO2 | IL1A | NFM | SLIK1 |
| A4 | CO3 | IL27A | NGF | SMOC1 |
| AACT | CO4A | IL6 | NICA | SODC |
| AATM | CO5 | IL6RA | NLGN3 | SODE |
| AFAM | CO6 | ITIH1 | NPTX1 | SORC1 |
| ALDOA | CO8B | ITIH5 | NPTX2 | SORC2 |
| AMBP | COCH | ITM2B | NPTXR | SORC3 |
| AMD | CRP | JAK1 | NPY | SPON1 |
| APLP2 | CSTN1 | KAIN | NRCAM | SPRL1 |
| APOA | CSTN3 | KCC2B | NRX1A | STX12 |
| APOA1 | CUTA | KI67 | NRX2A | SV2A |
| APOB | CYTC | KLK10 | NRX3A | SYNJ1 |
| APOC1 | DAG1 | KLK11 | NSG1 | SYT11 |
| APOD | DIAC | KLK12 | OSTP | TADBP |
| APOE | ENOG | KLK3 | PCD17 | TAU |
| B2MG | ENPP2 | KLK6 | PCMD1 | TCRG1 |
| B3GN1 | EXTL2 | KLK9 | PCSK1 | TEN3 |
| BACE1 | FABP5 | KLKB1 | PDIA3 | TGFB1 |
| BASP1 | FABP6 | KNG1 | PDYN | TGFB2 |
| BDNF | FABP7 | KPCZ | PEDF | TGFB3 |
| BTD | FABPH | KPYM | PGRP2 | TGON2 |
| C1QA | FABPI | L1CAM | PIMT | THRB |
| C1QB | FAM3C | LAMB2 | PLDX1 | TIMP1 |
| C3AR | FBLN1 | LFTY2 | PLMN | TNF14 |
| CA2D1 | FBLN3 | LPHN1 | PPN | TNFA |
| CAD13 | FETUA | LRC4B | PRDX1 | TNR1B |
| CADM3 | FMOD | LTBP2 | PRDX2 | TNR21 |
| CAH1 | GFAP | MIME | PRDX3 | TNR6 |
| CATA | GLNA | MMP2 | PRDX4 | TRBM |
| CATD | GOGB1 | MMP9 | PRDX5 | TRFE |
| CATL1 | GOLM1 | MMRN2 | PRDX6 | TRFM |
| CCKN | GRIA4 | MOG | PTGDS | TTHY |
| CCL25 | HBA | MTHR | PTPRD | UBB |
| CD14 | HBB | MUC18 | PTPRN | UCHL1 |
| CD59 | HEMO | NBL1 | PVRL1 | VASN |
| CERU | HERC4 | NCAM1 | RIMS3 | VGF |
| CFAB | I18BP | NCAM2 | SAP | VTDB |
| CH3L1 | IBP2 | NCAN | SCG1 | X3CL1 |
| CLUS | IBP6 | NEGR1 | SCG2 | |

of 294 peptides had significant expression changes ($q \leq 0.05$) associated with increasing *APOE4* copy number (Table 3 and Fig. 1A). CSF levels of an *APOE4*-specific peptide (APOE.LGADMEDVR) were substantially higher in *APOE4* carriers versus non-carriers ($q = 6.53 \times 10^{-81}$), consistent with prior studies [37, 38]. Two peptides found in all *APOE* isoforms had elevated CSF expression with increasing *APOE4* copy number (APOE.LAVYQAGAR, $q = 0.027$; APOE.LGPLVEQGR, $q = 0.027$). CSF expression of an *APOE2*-specific peptide was found to decrease with higher *APOE4* gene dosage (APOE.CLAVYQAGAR, $q = 0.027$).

Table 3

Expression of the Top 20 Peptides and for all complement peptides/proteins by *APOE4* copy number in multivariate models accounting for age and gender (model 1). For full data set, see Supplementary File 1

Top 20 proteins/peptides whose expression differed as a function of APOE4 copy number, controlling for age, gender, and clinical status (normal, MCI or AD)

| Protein | UniProt ID | Peptide | Log Fold Change | Average Expression | <i>t</i> statistic | p | FDR Corrected p |
|---------|------------|-------------------------|-----------------|--------------------|--------------------|-------|-----------------|
| APOE | P02649 | LGADMEDVR | 3.594 | 9.193 | 27.105 | 0.000 | 0.000 |
| CRP | P02741 | ESDTSYVSLK | -0.633 | 15.228 | -4.074 | 0.000 | 0.009 |
| APOE | P02649 | LGPLVEQGR | 0.287 | 22.419 | 3.588 | 0.000 | 0.038 |
| APOE | P02649 | LAVYQAGAR | 0.292 | 25.410 | 3.421 | 0.001 | 0.053 |
| ALDOA | P04075 | ALQASALK | 0.121 | 19.168 | 3.105 | 0.002 | 0.077 |
| CH3L1 | P36222 | ILGQQVPYATK | 0.112 | 23.114 | 3.133 | 0.002 | 0.077 |
| CH3L1 | P36222 | SFTLASSETGVGAPISGPGIPGR | 0.112 | 18.225 | 3.107 | 0.002 | 0.077 |
| FABPH | P05413 | SIVTLDDGGK | 0.131 | 14.664 | 3.208 | 0.001 | 0.077 |
| ALDOA | P04075 | QLLLTADDR | 0.111 | 16.246 | 2.963 | 0.003 | 0.079 |
| AMBP | P02760 | FLYHK | -0.167 | 11.800 | -2.953 | 0.003 | 0.079 |
| APOE | P02649 | AATVGLAGQPLQER | 0.247 | 20.145 | 3.039 | 0.003 | 0.079 |
| APOE | P02649 | CLAVYQAGAR | -0.630 | 8.715 | -2.919 | 0.004 | 0.079 |
| CH3L1 | P36222 | VTIDSSYDIK | 0.109 | 21.303 | 2.915 | 0.004 | 0.079 |
| FABPH | P05413 | SLGVGFATR | 0.108 | 15.706 | 2.998 | 0.003 | 0.079 |
| KNG1 | P01042 | TVGSDFYFSFK | -0.185 | 15.118 | -2.898 | 0.004 | 0.079 |
| AMBP | P02760 | ETLLQDFR | -0.171 | 18.977 | -2.777 | 0.006 | 0.107 |
| A2GL | P02750 | DLLLPPQDLR | -0.148 | 25.898 | -2.701 | 0.007 | 0.127 |
| A2GL | P02750 | VAAGAFQGLR | -0.139 | 23.017 | -2.458 | 0.015 | 0.152 |
| AATC | P17174 | IVASTLSNPFLFEWWTGNVK | 0.086 | 12.727 | 2.517 | 0.012 | 0.152 |
| AATM | P00505 | FVTVQTSISGTGALR | 0.097 | 10.314 | 2.496 | 0.013 | 0.152 |

CSF Complement proteins/peptides expression as a function of APOE4 copy number, controlling for age, gender, and clinical status (normal, MCI or AD)

| Protein | UniProt ID | Peptide | Log Fold Change | Average Expression | <i>t</i> statistic | p | FDR Corrected p |
|---------|------------|----------------------------|-----------------|--------------------|--------------------|-------|-----------------|
| C1QB | P02746 | LEQGENVFLQATDK | -0.042 | 17.128 | -1.241 | 0.216 | 0.622 |
| C1QB | P02746 | VPGLYFYTYHASSR | -0.053 | 17.604 | -1.135 | 0.257 | 0.669 |
| CFAB | P00751 | DAQYAPGYDK | -0.134 | 16.401 | -2.435 | 0.016 | 0.152 |
| CFAB | P00751 | VSEADSSNADWVTK | -0.111 | 16.220 | -2.471 | 0.014 | 0.152 |
| CFAB | P00751 | YGLVTYATYPK | -0.134 | 22.532 | -2.474 | 0.014 | 0.152 |
| CO2 | P06681 | DFHINLFR | -0.065 | 18.691 | -1.232 | 0.219 | 0.623 |
| CO2 | P06681 | HAIILLTDGK | -0.070 | 15.695 | -1.625 | 0.105 | 0.412 |
| CO2 | P06681 | SSGQWQTPGATR | -0.077 | 15.922 | -1.689 | 0.092 | 0.377 |
| CO3 | P01024 | IHWESASLLR | -0.267 | 14.006 | -2.136 | 0.034 | 0.235 |
| CO3 | P01024 | TELRPGETLNVNFFLLR | -0.081 | 10.150 | -1.997 | 0.047 | 0.259 |
| CO4A | P0C0L4 | DHAVDLIQK | -0.038 | 22.142 | -0.745 | 0.457 | 0.858 |
| CO4A | P0C0L4 | GSFEFPVGDVAVSK | -0.035 | 25.297 | -0.611 | 0.541 | 0.879 |
| CO4A | P0C0L4 | LGQYASPTAK | -0.041 | 21.701 | -0.760 | 0.448 | 0.857 |
| CO4A | P0C0L4 | NVNFQK | -0.019 | 18.387 | -0.392 | 0.695 | 0.978 |
| CO4A | P0C0L4 | VLSLAQEQVGGVGSPEK | -0.049 | 19.989 | -1.039 | 0.300 | 0.734 |
| CO4A | P0C0L4 | VTASDPLDTLGSEGALSPGGVASLLR | -0.029 | 18.008 | -0.712 | 0.477 | 0.871 |
| CO5 | P01031 | DINYVNPVIK | -0.045 | 16.181 | -0.630 | 0.529 | 0.876 |
| CO5 | P01031 | TLLPVSKPEIR | -0.050 | 17.209 | -0.757 | 0.449 | 0.857 |
| CO5 | P01031 | VFQFLEK | -0.043 | 18.210 | -0.644 | 0.520 | 0.873 |
| CO6 | P13671 | ALNHLPLEYNSALYSR | -0.113 | 16.297 | -1.772 | 0.077 | 0.345 |
| CO6 | P13671 | SEYGAALAWEK | -0.118 | 15.760 | -2.072 | 0.039 | 0.240 |
| CO8B | P07358 | IPGIFELGISSQSDR | -0.057 | 14.476 | -0.761 | 0.447 | 0.857 |
| CO8B | P07358 | SDLEVAHYK | -0.082 | 13.090 | -1.342 | 0.181 | 0.571 |
| CO8B | P07358 | YEFILK | -0.077 | 18.883 | -1.263 | 0.208 | 0.610 |

Log Fold Change - log₂ fold change of peptide expression with each additional *APOE4* allele copy in multivariate models for all analyzed CSF samples; Average expression – average peptide expression in all analyzed CSF samples; *t* statistic – hypothesis test statistic estimating the mean peptide expression (population mean) from the sampling distribution for each peptide in all analyzed CSF samples.

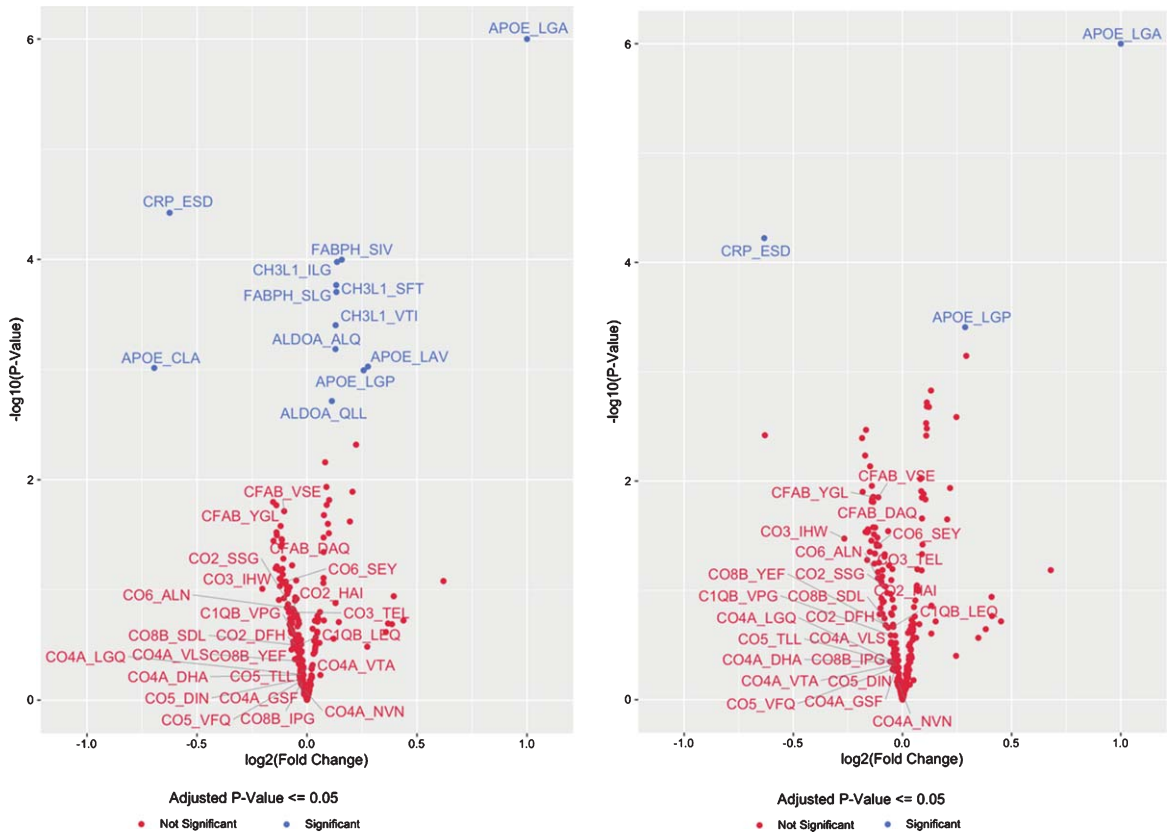


Fig. 1. Volcano Plot of CSF Protein/Peptide Expression by *APOE* genotype, for the top 20 proteins and the complement cascade proteins in model 1 (A), and for the top 20 proteins and the complement cascade proteins in model 2 (B).

Increasing *APOE4* allele copy number was associated with reduced expression of a peptide from the acute inflammatory marker C-reactive protein (CRP) ($q = 0.006$). Increasing *APOE4* copy number was also associated with increasing expression of peptides derived from the glycoprotein Chitinase 3-like protein 1 (CH3L1; also known as YKL-40) ($q < 0.05$), the cardiac injury biomarker heart-type fatty acid binding protein (FABPH) ($q < 0.05$), and the glycolytic enzyme fructose-bisphosphate aldolase A (ALDOA; $q < 0.05$).

There was a consistent inverse direction of association between *APOE4* copy number and CSF levels of all 24 peptides from all 8 complement pathway proteins measured (Table 3, Fig. 1A), although these effects were not significant for any individual complement protein-derived peptide ($p > 0.05$ for each, prior to multiple correction comparison). Nonetheless, the odds of this happening for 24 unrelated peptides by chance would be 1 over 2^{24} , or less than 1 in 16 million. Alternatively, since these 24 peptides were derived from 8 complement pathway proteins, the

odds of 8 proteins at random all showing consistently lower expression as a function of *APOE4* allele copy number, even if the change for each individual protein was not statistically significant, would be $1/2^8$, or a 1 out of 256 chance.

Model 2: APOE4-dependent CSF peptide changes and clinical status

Because *APOE4* is found in AD patients at disproportionately high frequencies compared to the general population, it is possible that the above findings reflect confounding by AD clinical status (and neurodegeneration) rather than changes directly related to increased *APOE4* copy number itself. Therefore, a second linear model was used that corrected for clinical status (normal control, MCI, or dementia due to AD) in addition to the items in model 1, to test for associations between *APOE4* copy number and CSF peptide expression levels. In this second model, only 3 of 294 peptides had statistically significant *APOE4* copy

number-related changes in CSF expression levels (Table 4 and Fig. 1B). Increasing *APOE4*-copy number was associated with increased expression of the *APOE4*-specific peptide (LGADMEDVR) ($q < 0.01$) and decreased expression the CRP-derived peptide (ESDTSYVSLK) ($q < 0.01$). A pan-APOE peptide (LGPLVEQGR) had increased expression associated with increased *APOE4* copy number in this model ($q = 0.038$). CH3L1 (YKL-40)-, FABPH-, and ALDOA-derived peptides that showed significant *APOE4*-copy number-related changes in expression in model 1 (above) no longer remained statistically significant after correcting for disease status and multiple comparisons, although there was still a trend toward increased CSF protein expression for each ($q = 0.077$, $q = 0.077$, and $q = 0.079$, respectively). As in the first model (not controlling for AD clinical status), none of the 24 complement protein-derived peptides demonstrated statistically significant differences as a function of *APOE4* copy number. Yet, as in the first model, in this model there was a consistent inverse direction of association between *APOE4* copy number and expression of all 24 peptides from all 8 different complement proteins measured (Table 4). Although none of these inverse associations were statistically significant on their own ($p > 0.05$ for each, prior to multiple comparison) the odds of 24 unrelated peptides all showing this pattern of decreased expression by chance would be less than 1 over 2^{24} , or less than 1 in 16 million. Alternatively, all 24 of these peptides were derived from 8 complement pathway proteins. Thus the odds of 8 proteins all showing lower expression levels as a function of *APOE4* allele copy number, even if the change in each individual protein was not statistically significant at $p < 0.05$, would be $1/2^8$, or a 1 out of 256 chance.

Model 3: APOE4-dependent CSF peptide changes and CSF tau levels

To determine whether *APOE4*-dependent CSF proteomic changes were independent of changes in CSF tau levels (a non-specific indicator of neurodegeneration [39]), we constructed a third model for associations between *APOE4* copy number and CSF peptide expression levels, correcting for CSF tau levels, age, and gender. In this third model, 183 of 294 peptides from 132 proteins had statistically significant *APOE4* copy number-related changes in CSF expression levels (Table 5, Supplementary File 2). Similar to the findings in models 1 and 2, in model 3 increasing *APOE4* copy number was

associated with increased expression of the *APOE4*-specific peptide (LGADMEDVR) ($q = 1.5 \times 10^{-75}$) and decreased expression of the CRP-derived peptide (ESDTSYVSLK) ($q = 0.0006$; Supplementary File 2). CH3L1 (YKL-40)-, FABPH-, and ALDOA-derived peptides that showed significant *APOE4* copy number-related changes in expression in model 1 (above) no longer remained statistically significant after correcting for CSF tau levels in model 3 ($q > 0.05$ for each; Supplementary File 2).

Four peptides from the complement cascade proteins C1QB, CFAB and CO2 showed significantly reduced expression as a function of increasing *APOE4* allele count in model 3 ($q < 0.05$ for each; Table 5). There was also a consistently inverse direction of association between *APOE4* copy number and CSF levels for the 20 other peptides from all 8 complement proteins measured (Table 5), although these effects were not statistically significant after multiple comparison correction ($q > 0.05$ for each).

Model 4: APOE4-dependent CSF peptide changes and CSF p-tau levels

To determine whether *APOE4*-dependent CSF proteomic changes were independent of changes in CSF p-tau levels (a more specific indicator of AD-related neurodegeneration [39]), we constructed a fourth model for associations between *APOE4* copy number and CSF peptide expression levels, correcting for CSF p-tau levels, age, and gender. In this fourth model, 184 of 294 peptides from 132 proteins had statistically significant *APOE4* copy number-related changes in CSF expression levels (Table 6). Similar to the findings in models 1 and 2, in model 3 increasing *APOE4*-copy number was associated with increased expression of the *APOE4*-specific peptide (LGADMEDVR) ($q = 4.1 \times 10^{-74}$) and decreased expression of the CRP-derived peptide (ESDTSYVSLK) ($q = 0.00094$). As in model 3 (controlling for CSF tau levels), CH3L1 (YKL-40)-, FABPH-, and ALDOA-derived peptides that showed significant *APOE4*-copy number-related changes in expression observed in model 1 no longer remained statistically significant after correcting for CSF p-tau levels ($q > 0.05$ for each; Supplementary File 2).

Three peptides from the complement cascade proteins C1QB, CFAB, and CO2 showed significantly reduced expression as a function of increasing *APOE4* allele count ($q < 0.05$ for each) in model 4. There was also a consistently inverse direction of

Table 4

Expression of the Top 20 Peptides and for all complement peptides/proteins by *APOE4* copy number in multivariate models accounting for age and gender and clinical status (Model 2). For full data set, see Supplementary File 1

Top 20 proteins/peptides whose expression differed as a function of APOE4 copy number, controlling for age, gender, and clinical status (normal, MCI or AD)

| Protein | UniProt ID | Peptide | Log Fold Change | Average Expression | <i>t</i> statistic | p | FDR Corrected p |
|---------|------------|-------------------------|-----------------|--------------------|--------------------|-------|-----------------|
| APOE | P02649 | LGADMEDVR | 3.594 | 9.193 | 27.105 | 0.000 | 0.000 |
| CRP | P02741 | ESDTSYVSLK | -0.633 | 15.228 | -4.074 | 0.000 | 0.009 |
| APOE | P02649 | LGPLVEQGR | 0.287 | 22.419 | 3.588 | 0.000 | 0.038 |
| APOE | P02649 | LAVYQAGAR | 0.292 | 25.410 | 3.421 | 0.001 | 0.053 |
| ALDOA | P04075 | ALQASALK | 0.121 | 19.168 | 3.105 | 0.002 | 0.077 |
| CH3L1 | P36222 | ILGQQVPYATK | 0.112 | 23.114 | 3.133 | 0.002 | 0.077 |
| CH3L1 | P36222 | SFTLASSETGVGAPISGPGIPGR | 0.112 | 18.225 | 3.107 | 0.002 | 0.077 |
| FABPH | P05413 | SIVTLDGGK | 0.131 | 14.664 | 3.208 | 0.001 | 0.077 |
| ALDOA | P04075 | QLLLTADDR | 0.111 | 16.246 | 2.963 | 0.003 | 0.079 |
| AMBP | P02760 | FLYHK | -0.167 | 11.800 | -2.953 | 0.003 | 0.079 |
| APOE | P02649 | AATVGLAGQPLQER | 0.247 | 20.145 | 3.039 | 0.003 | 0.079 |
| APOE | P02649 | CLAVYQAGAR | -0.630 | 8.715 | -2.919 | 0.004 | 0.079 |
| CH3L1 | P36222 | VTIDSSYDIK | 0.109 | 21.303 | 2.915 | 0.004 | 0.079 |
| FABPH | P05413 | SLGVGFATR | 0.108 | 15.706 | 2.998 | 0.003 | 0.079 |
| KNG1 | P01042 | TVGSDTFYSFK | -0.185 | 15.118 | -2.898 | 0.004 | 0.079 |
| AMBP | P02760 | ETLLQDFR | -0.171 | 18.977 | -2.777 | 0.006 | 0.107 |
| A2GL | P02750 | DLLLPPQDLR | -0.148 | 25.898 | -2.701 | 0.007 | 0.127 |
| A2GL | P02750 | VAAGAFQGLR | -0.139 | 23.017 | -2.458 | 0.015 | 0.152 |
| AATC | P17174 | IVASTLSNPFLFEWWTGNVK | 0.086 | 12.727 | 2.517 | 0.012 | 0.152 |
| AATM | P00505 | FVTVQTIISGTGALR | 0.097 | 10.314 | 2.496 | 0.013 | 0.152 |

CSF Complement proteins/peptides expression as a function of APOE4 copy number, controlling for age, gender, and clinical status (normal, MCI or AD)

| Protein | UniProt ID | Peptide | Log Fold Change | Average Expression | <i>t</i> statistic | p | FDR Corrected p |
|---------|------------|----------------------------|-----------------|--------------------|--------------------|-------|-----------------|
| C1QB | P02746 | LEQGENVFLQATDK | -0.042 | 17.128 | -1.241 | 0.216 | 0.622 |
| C1QB | P02746 | VPGLYFYFYHASSR | -0.053 | 17.604 | -1.135 | 0.257 | 0.669 |
| CFAB | P00751 | DAQYAPGYDK | -0.134 | 16.401 | -2.435 | 0.016 | 0.152 |
| CFAB | P00751 | VSEADSSNADWVTK | -0.111 | 16.220 | -2.471 | 0.014 | 0.152 |
| CFAB | P00751 | YGLVTYATYPK | -0.134 | 22.532 | -2.474 | 0.014 | 0.152 |
| CO2 | P06681 | DFHINLFR | -0.065 | 18.691 | -1.232 | 0.219 | 0.623 |
| CO2 | P06681 | HAIILLTDGK | -0.070 | 15.695 | -1.625 | 0.105 | 0.412 |
| CO2 | P06681 | SSGQWQTPGATR | -0.077 | 15.922 | -1.689 | 0.092 | 0.377 |
| CO3 | P01024 | IHWESASLLR | -0.267 | 14.006 | -2.136 | 0.034 | 0.235 |
| CO3 | P01024 | TELRPGETLNVNFFLLR | -0.081 | 10.150 | -1.997 | 0.047 | 0.259 |
| CO4A | P0C0L4 | DHAVDLIQK | -0.038 | 22.142 | -0.745 | 0.457 | 0.858 |
| CO4A | P0C0L4 | GSFEFVPGDAVSK | -0.035 | 25.297 | -0.611 | 0.541 | 0.879 |
| CO4A | P0C0L4 | LGQYASPTAK | -0.041 | 21.701 | -0.760 | 0.448 | 0.857 |
| CO4A | P0C0L4 | NVNFQK | -0.019 | 18.387 | -0.392 | 0.695 | 0.978 |
| CO4A | P0C0L4 | VLSLAQEQVGGVGSPEK | -0.049 | 19.989 | -1.039 | 0.300 | 0.734 |
| CO4A | P0C0L4 | VTASDPLDTLGSEGALSPGGVASLLR | -0.029 | 18.008 | -0.712 | 0.477 | 0.871 |
| CO5 | P01031 | DINYVNPVIK | -0.045 | 16.181 | -0.630 | 0.529 | 0.876 |
| CO5 | P01031 | TLLPVSKPEIR | -0.050 | 17.209 | -0.757 | 0.449 | 0.857 |
| CO5 | P01031 | VFQFLEK | -0.043 | 18.210 | -0.644 | 0.520 | 0.873 |
| CO6 | P13671 | ALNHLPLEYNSALYSR | -0.113 | 16.297 | -1.772 | 0.077 | 0.345 |
| CO6 | P13671 | SEYGAALAWEK | -0.118 | 15.760 | -2.072 | 0.039 | 0.240 |
| CO8B | P07358 | IPGIFELGISSQSDR | -0.057 | 14.476 | -0.761 | 0.447 | 0.857 |
| CO8B | P07358 | SDLEVAHYK | -0.082 | 13.090 | -1.342 | 0.181 | 0.571 |
| CO8B | P07358 | YEFILK | -0.077 | 18.883 | -1.263 | 0.208 | 0.610 |

Log Fold Change - log₂ fold change of peptide expression with each additional *APOE4* allele copy in multivariate models for all analyzed CSF samples; Average expression – average peptide expression in all analyzed CSF samples; *t* statistic – hypothesis test statistic estimating the mean peptide expression (population mean) from the sampling distribution for each peptide in all analyzed CSF samples.

Table 5

Expression for the Top 20 Peptides and for all complement peptides/proteins by *APOE4* copy number in multivariate models accounting for age, gender and CSF tau levels (Model 3). For full data set, see Supplementary File 2

| <i>Proteins/peptides whose expression differed as a function of APOE4 copy number, controlling for age, gender, and CSF tau level (in order of significance).</i> | | | | | | | |
|---|------------|----------------------------|-----------------|--------------------|--------------------|-------|-----------------|
| Protein | UniProt ID | Peptide | Log Fold Change | Average Expression | <i>t</i> statistic | p | FDR Corrected p |
| APOE | P02649 | LGADMEDVR | 3.365 | 9.208 | 26.640 | 0.000 | 0.000 |
| A4 | P05067 | LVFFAEDVGSNK | -0.166 | 18.133 | -5.315 | 0.000 | 0.000 |
| NCAM2 | O15394 | IIELSQTAK | -0.171 | 19.631 | -5.342 | 0.000 | 0.000 |
| VGF | O15240 | AYQGVAAFPFK | -0.254 | 17.733 | -5.299 | 0.000 | 0.000 |
| AMD | P19021 | IVQFSPSGK | -0.185 | 21.247 | -4.964 | 0.000 | 0.000 |
| CA2D1 | P54289 | FVVTDDGTR | -0.187 | 20.267 | -4.943 | 0.000 | 0.000 |
| CMGA | P10645 | YPGPQAEGDSEGLSQGLVDR | -0.237 | 15.851 | -5.008 | 0.000 | 0.000 |
| IGSF8 | Q969P0 | LQGDVVLLK | -0.133 | 18.301 | -4.885 | 0.000 | 0.000 |
| IGSF8 | Q969P0 | VVAGEVQVQR | -0.119 | 19.050 | -4.910 | 0.000 | 0.000 |
| NBL1 | P41271 | LALFPDK | -0.141 | 26.936 | -4.998 | 0.000 | 0.000 |
| SCG2 | P13521 | VLEYLNQEK | -0.202 | 20.916 | -4.897 | 0.000 | 0.000 |
| VGF | O15240 | NSEPQDEGELFQGVDPDR | -0.252 | 19.459 | -4.923 | 0.000 | 0.000 |
| VGF | O15240 | THLGEALAPLSK | -0.244 | 16.748 | -4.919 | 0.000 | 0.000 |
| FAM3C | P84101 | SPFEQHIK | -0.159 | 19.027 | -4.815 | 0.000 | 0.000 |
| AMD | P19021 | IPVDEEAFVIDFKPR | -0.167 | 17.376 | -4.735 | 0.000 | 0.000 |
| AMD | P19021 | NGQWTLIGR | -0.180 | 16.249 | -4.725 | 0.000 | 0.000 |
| CA2D1 | P54289 | TASGVNQLVDIYEK | -0.171 | 13.242 | -4.712 | 0.000 | 0.000 |
| CYTC | P01034 | ALDFAVGEYNK | -0.114 | 34.102 | -4.717 | 0.000 | 0.000 |
| NPTX2 | P47972 | TESTLNALLQR | -0.282 | 10.581 | -4.723 | 0.000 | 0.000 |
| CAD13 | P55290 | YEVSSPYFK | -0.161 | 22.725 | -4.657 | 0.000 | 0.000 |
| <i>CSF Complement proteins/peptides expression as a function of APOE4 copy number, controlling for age, gender, and tau</i> | | | | | | | |
| Protein | UniProt ID | Peptide | Log Fold Change | Average Expression | <i>t</i> statistic | p | FDR Corrected p |
| C1QB | P02746 | LEQGENVFLQATDK | -0.071 | 17.131 | -2.124 | 0.034 | 0.055 |
| C1QB | P02746 | VPLYLYFTYHASSR | -0.132 | 17.608 | -2.921 | 0.004 | 0.008 |
| CFAB | P00751 | DAQYAPGYDK | -0.111 | 16.394 | -2.015 | 0.045 | 0.068 |
| CFAB | P00751 | VSEADSSNADWVTK | -0.055 | 16.211 | -1.274 | 0.204 | 0.242 |
| CFAB | P00751 | YGLVTYATYPK | -0.124 | 22.526 | -2.270 | 0.024 | 0.039 |
| CO2 | P06681 | DFHINLFR | -0.088 | 18.688 | -1.670 | 0.096 | 0.129 |
| CO2 | P06681 | HAIILLTDGK | -0.101 | 15.692 | -2.360 | 0.019 | 0.032 |
| CO2 | P06681 | SSGQWQTPGATR | -0.102 | 15.920 | -2.228 | 0.027 | 0.043 |
| CO3 | P01024 | IHWESASLLR | -0.254 | 13.995 | -2.022 | 0.044 | 0.067 |
| CO3 | P01024 | TELRPGETLNVNFLLR | -0.083 | 10.147 | -2.028 | 0.044 | 0.067 |
| CO4A | P0C0L4 | DHAVDLIQK | -0.087 | 22.140 | -1.731 | 0.085 | 0.117 |
| CO4A | P0C0L4 | GSFEFPVGDVASK | -0.098 | 25.295 | -1.774 | 0.077 | 0.109 |
| CO4A | P0C0L4 | LGQYASPTAK | -0.106 | 21.701 | -2.052 | 0.041 | 0.064 |
| CO4A | P0C0L4 | NVNFQK | -0.071 | 18.386 | -1.490 | 0.137 | 0.176 |
| CO4A | P0C0L4 | VLSLAQEQVGGSPK | -0.062 | 19.985 | -1.317 | 0.189 | 0.229 |
| CO4A | P0C0L4 | VTASDPLDTLGSEGALSPGGVASLLR | -0.067 | 18.006 | -1.659 | 0.098 | 0.131 |
| CO5 | P01031 | DINYVNPVIK | -0.096 | 16.181 | -1.346 | 0.179 | 0.220 |
| CO5 | P01031 | TLLPVSKPEIR | -0.076 | 17.208 | -1.141 | 0.255 | 0.293 |
| CO5 | P01031 | VFQFLEK | -0.070 | 18.209 | -1.053 | 0.293 | 0.328 |
| CO6 | P13671 | ALNHLPLEYNSALYSR | -0.112 | 16.292 | -1.752 | 0.081 | 0.113 |
| CO6 | P13671 | SEYGAALAWEK | -0.104 | 15.755 | -1.817 | 0.070 | 0.102 |
| CO8B | P07358 | IPGIFELGISSQSDR | -0.045 | 14.471 | -0.595 | 0.552 | 0.584 |
| CO8B | P07358 | SDLEVAHYK | -0.055 | 13.083 | -0.883 | 0.378 | 0.413 |
| CO8B | P07358 | YEFILK | -0.045 | 18.876 | -0.727 | 0.468 | 0.499 |

association between *APOE4* copy number and CSF levels for the 21 other peptides from all 8 complement proteins measured (Table 6), although these effects were not statistically significant after multiple comparison correction in model 4 ($q > 0.05$ for each).

Model 5: APOE4-dependent CSF peptide changes and CSF A β levels

To determine whether *APOE4*-dependent CSF proteomic changes were independent of changes in CSF A β levels, we constructed a fifth model for

Table 6

Expression of the Top 20 Indicated Peptides and for all complement peptides/proteins by *APOE4* copy number in multivariate models accounting for age, gender and CSF p-tau levels (model 4). For full data set, see Supplementary File 2

| <i>Proteins/peptides whose expression differed as a function of APOE4 copy number, controlling for age, gender, and ptau</i> | | | | | | | |
|--|------------|---------------------------|-----------------|--------------------|--------------------|-------|-----------------|
| Protein | UniProt ID | Peptide | Log Fold Change | Average Expression | <i>t</i> statistic | p | FDR Corrected p |
| APOE | P02649 | LGADMEDVR | 3.340 | 9.208 | 26.198 | 0.000 | 0.000 |
| A4 | P05067 | LVFFAEDVGSNK | -0.168 | 18.133 | -5.170 | 0.000 | 0.000 |
| NCAM2 | O15394 | IIELSQTTAK | -0.175 | 19.631 | -5.197 | 0.000 | 0.000 |
| VEGF | O15240 | AYQGVAAFPFK | -0.258 | 17.733 | -5.151 | 0.000 | 0.000 |
| AMD | P19021 | IVQFSPSGK | -0.190 | 21.247 | -4.896 | 0.000 | 0.000 |
| CA2D1 | P54289 | FVVTDGGITR | -0.192 | 20.267 | -4.878 | 0.000 | 0.000 |
| CMGA | P10645 | YPGPQAEGDSEGLSQLVDR | -0.245 | 15.851 | -4.931 | 0.000 | 0.000 |
| NBL1 | P41271 | LALFPDK | -0.143 | 26.936 | -4.855 | 0.000 | 0.000 |
| VEGF | O15240 | NSEPQDEGELFQGVDPDR | -0.258 | 19.459 | -4.857 | 0.000 | 0.000 |
| FAM3C | P84101 | SPFEQHIK | -0.165 | 19.027 | -4.768 | 0.000 | 0.000 |
| IGSF8 | Q969P0 | VVAGEVQVQR | -0.121 | 19.050 | -4.784 | 0.000 | 0.000 |
| SCG2 | P13521 | VLEYLNQEK | -0.207 | 20.916 | -4.822 | 0.000 | 0.000 |
| VEGF | O15240 | THLGEALAPLSK | -0.249 | 16.748 | -4.799 | 0.000 | 0.000 |
| IGSF8 | Q969P0 | LQGDVAVLK | -0.135 | 18.301 | -4.735 | 0.000 | 0.000 |
| AMD | P19021 | IPVDEEAFVIDFKPR | -0.170 | 17.376 | -4.623 | 0.000 | 0.000 |
| AMD | P19021 | NGQWTLIGR | -0.183 | 16.249 | -4.615 | 0.000 | 0.000 |
| CA2D1 | P54289 | TASGVNQLVDIYEK | -0.175 | 13.242 | -4.641 | 0.000 | 0.000 |
| CAD13 | P55290 | YEVSSPYFK | -0.167 | 22.725 | -4.601 | 0.000 | 0.000 |
| CMGA | P10645 | SEALAVDVGAGKPGAEEAQDPEGK | -0.216 | 17.989 | -4.597 | 0.000 | 0.000 |
| CMGA | P10645 | SGEATDGARQPALPEPMQESK | -0.206 | 19.085 | -4.642 | 0.000 | 0.000 |
| <i>CSF Complement proteins/peptides expression as a function of APOE4 copy number, controlling for age, gender, and ptau</i> | | | | | | | |
| Protein | UniProt ID | Peptide | Log Fold Change | Average Expression | <i>t</i> statistic | p | FDR Corrected p |
| C1QB | P02746 | LEQGENVFLQATDK | -0.069 | 17.131 | -2.054 | 0.041 | 0.064 |
| C1QB | P02746 | VPGLYFYFTYHASSR | -0.130 | 17.608 | -2.813 | 0.005 | 0.010 |
| CFAB | P00751 | DAQYAPGYDK | -0.107 | 16.394 | -1.925 | 0.055 | 0.083 |
| CFAB | P00751 | VSEADSSNADWVTK | -0.048 | 16.211 | -1.102 | 0.271 | 0.319 |
| CFAB | P00751 | YGLVYATYPK | -0.119 | 22.526 | -2.169 | 0.031 | 0.049 |
| CO2 | P06681 | DFHINLFR | -0.086 | 18.688 | -1.610 | 0.109 | 0.146 |
| CO2 | P06681 | HAIILLTDGK | -0.100 | 15.692 | -2.293 | 0.023 | 0.038 |
| CO2 | P06681 | SSGQWQTPGATR | -0.099 | 15.920 | -2.137 | 0.033 | 0.053 |
| CO3 | P01024 | IHWESASLLR | -0.249 | 13.995 | -1.966 | 0.050 | 0.077 |
| CO3 | P01024 | TELRPGETLNVNFLLR | -0.082 | 10.147 | -1.982 | 0.048 | 0.074 |
| CO4A | POC0L4 | DHAVDLIQK | -0.080 | 22.140 | -1.563 | 0.119 | 0.157 |
| CO4A | POC0L4 | GSFEFPVGDVASK | -0.091 | 25.295 | -1.596 | 0.112 | 0.149 |
| CO4A | POC0L4 | LGQYASPTAK | -0.099 | 21.701 | -1.866 | 0.063 | 0.092 |
| CO4A | POC0L4 | NVNFQK | -0.064 | 18.386 | -1.314 | 0.190 | 0.234 |
| CO4A | POC0L4 | VLSLAQEQVGGSPK | -0.053 | 19.985 | -1.114 | 0.266 | 0.314 |
| CO4A | POC0L4 | VTASDPLDTLGSEGLSPGGVASLLR | -0.061 | 18.006 | -1.481 | 0.140 | 0.179 |
| CO5 | P01031 | DINYVNPVIK | -0.092 | 16.181 | -1.264 | 0.207 | 0.251 |
| CO5 | P01031 | TLLPVSKPEIR | -0.070 | 17.208 | -1.043 | 0.298 | 0.345 |
| CO5 | P01031 | VFQFLEK | -0.065 | 18.209 | -0.965 | 0.335 | 0.378 |
| CO6 | P13671 | ALNHLPLEYNSALYSR | -0.109 | 16.292 | -1.689 | 0.092 | 0.132 |
| CO6 | P13671 | SEYGAAALAWK | -0.101 | 15.755 | -1.744 | 0.082 | 0.119 |
| CO8B | P07358 | IPGIFELGISSQSDR | -0.036 | 14.471 | -0.478 | 0.633 | 0.660 |
| CO8B | P07358 | SDLEVAHYK | -0.049 | 13.083 | -0.781 | 0.435 | 0.472 |
| CO8B | P07358 | YEFILK | -0.038 | 18.876 | -0.613 | 0.541 | 0.570 |

associations between *APOE4* copy number and CSF peptide expression levels, correcting for CSF A β levels as well as age and gender. In this model, 176 of 294 peptides had statistically significant *APOE4* copy number-related changes in CSF expression levels (Supplementary File 2). Increasing *APOE4*-copy number was associated with increased expression

of the *APOE4*-specific peptide (LGADMEDVR) ($q = 1.89 \times 10^{-62}$) and decreased expression of the CRP-derived peptide (ESDTSYVSLK) ($q < 0.045$) after controlling for CSF A β levels. Increasing *APOE4* copy number was associated with increased expression of a pan-APOE peptide (LGPLVEQGR) in this model ($q = 1.69 \times 10^{-7}$). As in models 1 and

Table 7

Expression of the Top 20 Indicated Peptides and for all complement peptides/proteins by *APOE4* copy number in multivariate models accounting for age, gender and CSF A β levels (Model 5). For full data set, see Supplementary File 2

| <i>Top 20 Proteins/Peptides whose Expression differed as function of APOE4 copy number, controlling for age, gender, and Aβ</i> | | | | | | | |
|--|------------|----------------------------|-----------------|--------------------|--------------------|-------|-----------------|
| Protein | UniProt ID | Peptide | Log Fold Change | Average Expression | <i>t</i> statistic | p | FDR Corrected p |
| APOE | P02649 | LGADMEDVR | 3.664 | 9.224 | 23.026 | 0.000 | 0.000 |
| APOE | P02649 | LGPLVEQGR | 0.565 | 22.385 | 6.309 | 0.000 | 0.000 |
| APOE | P02649 | LAVYQAGAR | 0.572 | 25.381 | 6.007 | 0.000 | 0.000 |
| APOE | P02649 | AATVGSLAGQPLQER | 0.515 | 20.108 | 5.631 | 0.000 | 0.000 |
| NRX1A | Q9ULB1 | ITTQITAGAR | 0.240 | 16.206 | 4.981 | 0.000 | 0.000 |
| AATM | P00505 | FVTVQTISGTGALR | 0.209 | 10.293 | 4.719 | 0.000 | 0.000 |
| NRX1A | Q9ULB1 | DLFIDGQSK | 0.209 | 16.186 | 4.688 | 0.000 | 0.000 |
| PCSK1 | P29120 | GEAAGAVQELAR | 0.245 | 21.388 | 4.676 | 0.000 | 0.000 |
| L1CAM | P32004 | LVLSDLHLLTQSQVR | 0.229 | 10.874 | 4.607 | 0.000 | 0.000 |
| CADM3 | Q8N126 | GNPVPQQYLWEK | 0.179 | 15.815 | 4.557 | 0.000 | 0.000 |
| AATC | P17174 | NLDYVATSIHEAVTK | 0.193 | 11.536 | 4.312 | 0.000 | 0.000 |
| ALDOA | P04075 | ALQASALK | 0.202 | 19.150 | 4.415 | 0.000 | 0.000 |
| ALDOA | P04075 | QLLLTADDR | 0.190 | 16.230 | 4.332 | 0.000 | 0.000 |
| CADM3 | Q8N126 | EGSVPLK | 0.158 | 16.142 | 4.328 | 0.000 | 0.000 |
| CADM3 | Q8N126 | SLVTVLGIPQKPIITGYK | 0.191 | 16.167 | 4.424 | 0.000 | 0.000 |
| KPYM | P14618 | LDIDSPITAR | 0.214 | 18.500 | 4.308 | 0.000 | 0.000 |
| NRX1A | Q9ULB1 | SDLYIGGVAK | 0.189 | 16.548 | 4.329 | 0.000 | 0.000 |
| PCSK1 | P29120 | ALAHLLAER | 0.242 | 19.035 | 4.329 | 0.000 | 0.000 |
| PCSK1 | P29120 | NSDPALGLDDDPDAPAAQLAR | 0.227 | 13.959 | 4.351 | 0.000 | 0.000 |
| PRDX1 | Q06830 | DISLSDYK | 0.185 | 14.594 | 4.377 | 0.000 | 0.000 |
| <i>CSF Complement Protein/Peptide Expression as a function of APOE4 copy number, controlling for age, gender, and Aβ</i> | | | | | | | |
| Protein | UniProt ID | Peptide | Log Fold Change | Average Expression | <i>t</i> statistic | p | FDR Corrected p |
| C1QB | P02746 | LEQGENVFLQATDK | 0.039 | 17.123 | 0.983 | 0.326 | 0.393 |
| C1QB | P02746 | VPGLYFYFYHASSR | 0.096 | 17.588 | 1.782 | 0.076 | 0.114 |
| CFAB | P00751 | DAQYAPGYDK | -0.009 | 16.383 | -0.132 | 0.895 | 0.904 |
| CFAB | P00751 | VSEADSSNADWVTK | -0.051 | 16.209 | -0.956 | 0.340 | 0.408 |
| CFAB | P00751 | YGLVTYATYPK | 0.007 | 22.512 | 0.119 | 0.905 | 0.911 |
| CO2 | P06681 | DFHINLFR | 0.085 | 18.670 | 1.381 | 0.168 | 0.232 |
| CO2 | P06681 | HAIILLTDGK | 0.050 | 15.673 | 1.008 | 0.315 | 0.384 |
| CO2 | P06681 | SSGQWQTPGATR | 0.054 | 15.901 | 1.026 | 0.306 | 0.378 |
| CO3 | P01024 | IHWESALLR | -0.032 | 13.985 | -0.215 | 0.830 | 0.853 |
| CO3 | P01024 | TELRPGETLNVNFFLLR | -0.011 | 10.138 | -0.227 | 0.821 | 0.847 |
| CO4A | P0C0L4 | DHAVDLIQK | 0.100 | 22.139 | 1.690 | 0.092 | 0.135 |
| CO4A | P0C0L4 | GSFEFPVGDVASK | 0.140 | 25.290 | 2.132 | 0.034 | 0.056 |
| CO4A | P0C0L4 | LGQYASPTAK | 0.122 | 21.693 | 1.975 | 0.049 | 0.079 |
| CO4A | P0C0L4 | NVNFQK | 0.100 | 18.384 | 1.776 | 0.077 | 0.115 |
| CO4A | P0C0L4 | VLSLAQEQQVGGSPK | 0.055 | 19.994 | 1.009 | 0.314 | 0.384 |
| CO4A | P0C0L4 | VTASDPLDTLGSEGALSPGGVASLLR | 0.066 | 18.007 | 1.380 | 0.169 | 0.232 |
| CO5 | P01031 | DINYVNPVIK | 0.241 | 16.153 | 2.987 | 0.003 | 0.007 |
| CO5 | P01031 | TLLPVSKPEIR | 0.207 | 17.184 | 2.768 | 0.006 | 0.012 |
| CO5 | P01031 | VFQFLEK | 0.216 | 18.187 | 2.874 | 0.004 | 0.010 |
| CO6 | P13671 | ALNHLPLEYNSALYSR | 0.054 | 16.276 | 0.731 | 0.465 | 0.526 |
| CO6 | P13671 | SEYGAALAWEK | 0.029 | 15.745 | 0.434 | 0.665 | 0.706 |
| CO8B | P07358 | IPGIFELGISSQSDR | 0.107 | 14.458 | 1.208 | 0.228 | 0.298 |
| CO8B | P07358 | SDLEVAHYK | 0.049 | 13.073 | 0.679 | 0.498 | 0.560 |
| CO8B | P07358 | YEFILK | 0.061 | 18.865 | 0.844 | 0.399 | 0.468 |

2, all five CH3L1-, FABPH-, and ALDOA-derived peptides also showed statistically significant *APOE4* copy number related increases in expression after correcting for CSF A β levels ($q < 0.05$ for each; Table 7).

In this model controlling for CSF A β levels (as well as age and sex), all three peptides from complement factor 5 (CO5) showed a statistically significant

increase in expression as a function of increasing *APOE4* copy number ($q < 0.05$ for each; Table 7). In contrast to the results from models 1–4, in this model (controlling for CSF A β levels) no consistent direction of association was seen between *APOE4* copy number and the other 21 complement-derived peptides (Table 7).

Table 8

Summary of Change Direction for Top proteins and Complement Pathway Proteins in Statistical Models 1 – 5. Up arrow indicates expression increase for the indicated protein as a function of increasing *APOE4* copy number in the given statistical model; down arrow indicates expression decrease for the indicated protein as a function of increasing *APOE4* copy number in the given statistical model

| Protein | UniProt ID | Peptide | Key functions | Statistical Model [#] | | | | |
|-----------------------------|------------|---|---|--------------------------------|----------------|----------------|----------------|----------------|
| | | | | 1 | 2 | 3 | 4 | 5 |
| CRP | P02741 | ESDTSYVSLK | Acute phase reactant [40]. Mediator of inflammatory and apoptotic processes, including activation of classical complement pathway [41], opsonization of atherosclerotic plaques [42]. | ↓ ² | ↓ ² | ↓ ³ | ↓ ³ | ↓ ¹ |
| CH3L1 | P36222 | ILGQQVPYATK VTIDSSYDIAK | Glycoprotein thought to modulate tissue remodeling, and angiogenesis, highly expressed in reactive astrocytes after acute and chronic neuroinflammation [63–65]. Also expressed by activated microglia. | ↑ | ↑ | ↑ | ↑ | ↑ |
| FABPH | P05413 | SIVTLDGGK SLGVGFATR | Lipid transporter regulates membrane composition and stability [71], may alter the activity of gamma-secretase or slow trans-membrane lipid transport to increase Aβ deposition in <i>APOE4</i> carriers [72]. | ↑ | ↑ | ↓ | ↓ | ↑ |
| ALDOA | P04075 | ALQASALK QLLLTADDR | Glycolytic enzyme that breaks down fructose 1-6-diphosphate; decreased glucose metabolism has been observed in the brains of patients with AD [73], likely due to oxidative stress that impairs ATP production, induces synaptic dysfunction, and causes neuronal death [74]. | ↑ | ↑ | ↓ | ↓ | ↑ ³ |
| Complement pathway proteins | | | | | | | | |
| C1QB | P02746 | LEQGENVFLQATDK VPGLYYFTYHASSR | Complement pathway activation and complement factor-dependent synaptic phagocytosis thought to represent a neurodegeneration mechanism in AD [82, 83] | ↓ | ↓ | ↓ | ↓ | ↑ |
| CFAB | P00751 | DAQYAPGYDK VSEADSSNADWVTK YGLVTYATYPK | | ↓ | ↓ | ↓ ² | ↓ ¹ | ↓ |
| CO2 | P06681 | DFHINLFR HAILLLTDGK SSGQWQTPGATR | ↓ | ↓ | ↓ ¹ | ↓ ¹ | ↑ | |
| CO3 | P01024 | IHWESASLLR TELRPGETLNVNFLLR | ↓ | ↓ | ↓ | ↓ | ↓ | |
| CO4A | P0C0L4 | DHAVDLIQK GSFEFPVGDVAVSK LGQYASPTAK NVNFQK VLSLAQEQVGGSPK VTASDPLDTLGSEK SPGGVASLLR | ↓ | ↓ | ↓ | ↓ | ↑ | |
| CO5 | P01031 | DINYVNPVIK TLLPVSKPEIR VFQFLEK | ↓ | ↓ | ↓ | ↓ | ↑ ² | |
| CO6 | P13671 | ALNHLPLEYNSALYSR SEYGAALAWEK | ↓ | ↓ | ↓ | ↓ | ↑ | |
| CO8B | P07358 | IPGIFELGISSQSDR SDLEVAHYK YEFILK | ↓ | ↓ | ↓ | ↓ | ↑ | |

[#]Statistical Models: Model 1: *APOE4*-dependent CSF peptide changes and age and gender. Model 2: *APOE4*-dependent CSF peptide changes and age, gender, and AD clinical status. Model 3: *APOE4*-dependent CSF peptide changes and age, gender, and CSF tau levels. Model 4: *APOE4*-dependent CSF peptide changes and age, gender, and CSF p-tau levels. Model 5: *APOE4*-dependent CSF peptide changes and age, gender, and CSF Aβ levels. ¹FDR corrected $p < 0.05$. ²FDR corrected $p < 0.01$. ³FDR corrected $p < 0.001$.

DISCUSSION

Here we found that increasing *APOE4* copy number was associated with decreased CSF CRP levels,

which remained significant even after controlling for AD clinical status (Model 2), CSF tau levels (Model 3), CSF p-tau levels (Model 4), and/or CSF Aβ levels (Model 5; see Table 8 for summary of changes

across models). In several models, we also found significant *APOE4* copy number-related increases in pan-APOE peptides, and *APOE4* copy number-related increases in peptides from CH3L1, FABPH, and ALDOA. Finally, except when controlling for CSF A β levels in model 5, in all other models a consistent inverse direction of association was observed between increasing *APOE4* copy number and CSF levels of all 8 complement proteins measured in this targeted proteomics dataset.

One of the most consistent findings across all five statistical models examined here was that increasing *APOE4* copy number was associated with reduced CSF levels of the CRP-derived peptide (ESDT-SYVSLK, see Table 8). CRP is an acute phase reactant [40] and acts as a mediator of inflammatory and apoptotic processes, including activation of the classical complement pathway [41] and opsonization of atherosclerotic plaques [42]. Recent prospective studies have found that elevated serum CRP levels in midlife may predict increased AD risk, though there is paradoxical shift in which CRP levels decline with advancing age and before clinical AD symptoms appear [43–45]. Further, reduced CRP levels in peripheral blood [46–49] and in CSF [50, 51] correlate with increased cognitive dysfunction and further AD progression in an *APOE4*-dependent manner. These findings fit well with our observation of a significant decrease in CSF CRP levels as a function of increasing *APOE4* copy number and suggests that reduced CRP levels may play a key biological role in *APOE4*-induced increased AD risk.

CRP is typically viewed as a marker of inflammation, so a straightforward interpretation of these results would be that increasing *APOE4* copy number is associated with decreased CNS inflammation, which could play a role in increased AD risk if *APOE4* leads to a reduction in inflammatory processes involved in clearing amyloid plaques or other neurotoxic pathology. Alternatively, although elevated CRP levels are typically viewed as indicative of active inflammation, low and low-normal CRP levels have been found in chronic inflammatory conditions with active disease such as lupus [52], rheumatoid arthritis [53], and inflammatory bowel disease [54, 55]. Thus, the reduced CSF CRP levels observed here may similarly reflect chronically increased inflammation within the CNS of *APOE4* carriers, which could play a role in neurodegeneration and AD risk. CRP has been also implicated in the early development of amyloid plaque formation, neuronal damage, and AD risk [53–56]. The decreased

CSF CRP levels observed here may reflect CRP deposition in A β plaques and neurofibrillary tangles, in which CRP might play a role in promoting the development of AD. Low CSF CRP levels could also reflect increased CRP consumption from opsonin-mediated glial phagocytosis in AD pathology, as previously suggested [62]. In this way, CSF CRP reductions might alternatively reflect a compensatory glial-dependent A β removal process in *APOE4* carriers. Future studies should thus determine whether lower CSF CRP levels in *APOE4* carriers represent a mechanism of neurodegeneration or a compensatory process against it, or even an *APOE4*-copy related process unrelated to the development of AD.

All five models studied here also demonstrated a strong positive correlation between *APOE4* copy number and CSF expression of the *APOE4* allele specific peptide LGADMEDVR [37, 38], which serves as a strong internal control for the validity of this dataset. We also found that increasing *APOE4* copy number was associated with increased CSF pan-APOE peptide levels when controlling for AD clinical status or CSF A β levels (Models 2 and 5, respectively) but not when controlling for either CSF tau or CSF p-tau levels (Models 3 and 4, respectively). Taken together, these results are consistent with a paradigm in which *APOE4* allele leads to increased CSF ApoE protein levels before and during the phase in which patients are developing brain A β pathology (as measured here by CSF A β levels). Then, as frank neurodegeneration begins (as evidenced by rising CSF tau and p-tau levels), there is no longer a significant increase in CSF ApoE protein levels as a function of increasing *APOE4* allele count. This could help explain conflicting prior studies on CSF ApoE protein levels in *APOE4* carriers [57–59], since it suggests that the relationship between *APOE4* copy number and CSF ApoE protein levels would change as neurodegeneration begins (i.e., as defined by increasing CSF tau and p-tau levels). Yet, it is unclear whether the increased AD risk in *APOE4* carriers is due to *APOE4* copy number-related increases in ApoE protein levels versus functional or structural changes in the *APOE4* allele-encoded ApoE protein. Indeed, it remains debated in the field to what extent *APOE4*-related increased AD risk represents a toxic gain of function(s) or a loss of protective function(s) (reviewed in [60]).

In models 1 and 5, but not in models 2–4 (Table 8), increasing *APOE4* copy number was also associated with increased expression of peptides derived from CH3L1 (also known as YKL-40) ($q < 0.05$),

FABPH ($q < 0.05$), and ALDOA ($q < 0.05$), as seen in another recent paper [61]. These findings suggest that *APOE4* copy number is associated with increased CSF CH3L1, FABPH, and CH3L1 levels independent of CSF A β level changes, but these increases dissipate after controlling for neurodegeneration (i.e., via rising CSF tau or p-tau levels) or by controlling for neurodegeneration-related clinical status (i.e., MCI or dementia).

Our finding of *APOE4*-related increases in CSF CH3L1 levels is corroborated by another recent study using ELISA assays that found increased CSF CH3L1 levels in *APOE4* carriers [62]. CH3L1 is a glycoprotein hypothesized to modulate tissue remodeling, and angiogenesis and is highly expressed in reactive astrocytes after acute and chronic neuroinflammation [63–65]. After traumatic brain injuries, *CH3L1* knockout mice demonstrated greater astrogliosis, immune cell infiltration, and neurologic impairment compared to wild-type mice, suggesting CH3L1 plays a role in regulating neuroinflammation [66]. Astrocytes are known regulators of neuroinflammation and oxidative stress in the nervous system, and CH3L1-expressing astrocytes have also been shown to cluster around A β plaques and vessels with β -amyloid angiopathy in AD [67]. Taken together, these findings and the data presented here suggest that CH3L1 may contribute to pathologic astrocyte activation in *APOE4* carriers while A β pathology is developing, prior to actual neurodegeneration.

Our finding of a positive correlation between *APOE4* copy number and CSF FABPH levels when controlling for A β levels, but not when controlling for CSF neurodegeneration markers (i.e., CSF tau and p-tau; Table 8), is consistent with prior studies suggesting a role for FABPH in AD [29, 37, 68–70]. FABPH is a lipid transporter that regulates membrane composition and stability [71], and may alter the activity of gamma-secretase or slow transmembrane lipid transport to increase A β deposition in *APOE4* carriers [72]. Indeed, prior studies have found that CSF FABPH levels in *APOE4* carriers are associated with low CSF A β levels and atrophy of the entorhinal cortex and other AD-vulnerable brain regions [69]. These findings indicate a potential role for FABPH in *APOE4*-mediated A β accumulation and later neurodegeneration. The findings presented here further support this idea, and further suggest that altered FABPH levels likely play a role in the development of early A β pathology in *APOE4* carriers, before CSF tau and p-tau levels begin to decline.

We also found that increasing *APOE4* copy number was associated with increased CSF ALDOA levels in models one and five (Table 8). ALDOA is a glycolytic enzyme that catalyzes the breakdown of fructose 1-6-diphosphate, which is interesting because decreased glucose metabolism has been observed in the brains of patients with AD [73], likely due to oxidative stress that impairs ATP production, induces synaptic dysfunction, and causes neuronal death [74]. A recent study has also proposed that CSF ALDOA levels are a sensitive and specific biomarker of cognitive impairment due to AD [75]. Thus, our finding of an *APOE4* copy number-dependent increase in CSF ALDOA levels may reflect altered glucose metabolism within the CNS of *APOE4* carriers, which has also been observed in AD. It is unclear whether increased ALDOA levels are part of the mechanism for impaired glucose metabolism in *APOE4* carriers and in AD, or whether these increased ALDOA levels reflect a secondary biologic compensation as the result of dysregulated glucose metabolism in *APOE4* carriers. As in the case of CH3L1 and ALDOA, the data presented here suggest that *APOE4* copy number is likely associated with increased CSF ALDOA levels while individuals are clinically normal, and either completely free of AD neuropathology or when they show CSF evidence of A β , but not tau or p-tau, pathology.

Further, while not statistically significant, there was a consistent inverse direction of association between *APOE4* copy number and expression of all 24 peptides from all 8 different complement proteins measured (Fig 1A, B) in model 1. These findings were present even after controlling for AD clinical status in model 2, suggesting that the lower complement levels seen as a function of *APOE4* copy number do not simply reflect confounding due to increased AD dementia frequency in *APOE4* carriers. Further, this consistent inverse direction of association between *APOE4* copy number and expression of all 24 peptides from all 8 different complement proteins measured here was also observed after controlling for CSF tau and p-tau levels in models 3 and 4, respectively. These findings suggest that these potential *APOE4*-related reductions in CSF complement levels are not due to confounding from neurodegeneration in *APOE4* carriers.

Nonetheless, this consistent inverse direction of association between *APOE4* copy number and CSF complement protein expression was not observed in model 5, which controlled for CSF A β levels. These findings would fit with a scenario in which

APOE4 leads to reductions in both CSF A β levels and CSF complement protein levels, and these *APOE4*-dependent reductions in CSF A β and complement levels are closely associated with one another (or co-linear). In this case, increasing *APOE4* copy number would be expected to be associated with reduced complement levels in all situations except when controlling for falling CSF A β levels. This framework is consistent with the finding that complement proteins deposit into A β plaques [76, 77], which would offer a potential biochemical explanation for a co-linear relationship between reductions in CSF A β and complement levels as a function of increasing *APOE4* copy number.

The finding that *APOE4* copy number may be associated with decreased CSF complement protein levels is supported by two other recent studies that also found lower CSF complement protein levels in *APOE4* carriers [61, 78]. While the absolute magnitude of the reduction in each complement-protein derived peptide was modest, the chance that the level of 24 peptides (or 8 proteins) would all decrease due to chance alone is extremely low, suggesting that this likely represents a true biological finding. These lower complement protein levels could represent either decreased complement factor transcription/translation or increased degradation in *APOE4* carriers. The former possibility is unlikely, though, because prior work has shown that *APOE4* is not associated with alterations in the transcription or translation of complement pathway proteins [79]. These data therefore suggest that increasing *APOE4* copy number may be associated with increased complement pathway protein degradation in the CNS.

Complement protein degradation can be caused by complement cascade activation, which involves cleavage and degradation of complement proteins [80]. Thus, the relationship between increasing *APOE4*-copy number and potential reductions in CSF complement protein levels may reflect increased complement pathway activation. Recent work suggests that the ApoE protein is a negative regulator of complement pathway activation [81]. Taken together with our results, this raises the possibility that the *APOE4* allele results in reduced inhibition (i.e., disinhibition) of the complement pathway, thus resulting in *APOE4* copy number-dependent reductions in CSF levels of complement pathway proteins (due to complement degradation).

Complement pathway activation and complement factor-dependent synaptic phagocytosis is thought to represent a neurodegeneration mechanism in AD [78,

82]; thus, our results raise the possibility that *APOE4* may contribute to AD risk by increasing complement pathway activation and resultant synaptic phagocytosis and neurodegeneration. Overall, even though the *APOE4*-related reductions in CSF complement protein levels seen here were generally not statistically significant after multiple comparison correction, the convergence of our human findings with data from cellular [79] and mouse models [83] suggest that further studies are warranted on the relationship between *APOE4* copy number and CSF complement protein levels.

This work has several limitations. First, the data set studied here included very few African-American *APOE4* allele carriers, raising the question of how well these data may generalize to African-American or other non-Caucasian populations. Future work should study the effects of the *APOE4* allele on the CSF proteome in other racial and ethnic populations to develop a broader understanding of how *APOE4* may increase AD risk. Second, the data analyzed here were originally obtained to study CSF proteomic correlates of dementia due to AD or MCI [29], rather than to study the effects of *APOE4* copy number on the CSF proteome. Although we controlled for clinical status in model 2, the relatively smaller number of study patients within each clinical status cohort (i.e., normal, MCI, or dementia due to AD) likely limited statistical power to detect effects of *APOE4* on the CSF proteome itself. Thus, future studies on this topic should focus on larger clinically homogenous study populations (i.e., all cognitively normal individuals, or all individuals with dementia due to AD) to reduce variance within each genotype group, and to improve statistical power. Third, the data reported here were from a targeted proteomic platform that only measured 222 proteins total, including just 8 of the over 30 proteins in the full complement pathway [84]. Future studies should focus on quantitating the CSF levels of a broader array of proteins including all complement pathway protein to develop a more complete understanding of the relationship between *APOE4* allele copy number and the classical, lectin and alternative complement cascades and other protein pathways.

Nonetheless, the data presented here provide strong support for the hypothesis that the increased AD risk in *APOE4* carriers is related to changes within CRP-related biological processes, and to changes in biological mechanisms involving CH3L1, FABPH, ALDOA, and the complement pathway. The results from our five different statistical models also

demonstrate key differences across the neuropathologic phases of preclinical AD in the CSF proteomic correlates of *APOE4* copy number. Thus, future CSF proteomic studies designed to identify the earliest mechanisms by which *APOE4* contributes to AD risk may need to focus on patients without significant A β or neurodegenerative pathology.

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SUPPLEMENTARY MATERIAL

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