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FEATURED ARTICLE

Apolipoprotein B is a novel marker for early tau pathology in Alzheimer's disease

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A portion of the data used in preparation of this article was obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.ucla.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.ucla.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

Abstract

Introduction: We examine the role of brain apolipoprotein B (apoB) as a putative marker of early tau pathology and cognitive decline.

Methods: Cerebrospinal fluid (CSF) samples from cognitively normal and Alzheimer's disease (AD) participants were collected to measure protein levels of apoB and AD biomarkers amyloid beta (Aβ), t-tau and p-tau, as well as synaptic markers GAP43, SYNAPTOTAGMIN-1, synaptosome associated protein 25 (SNAP-25), and NEUROGRANIN. CSF apoB levels were contrasted with positron emission tomography (PET) scan measures of Aβ (18F-NAV4694) and Tau (flortaucipir) along with cognitive assessment alterations over 6 to 8 years.

Results: CSF apoB levels were elevated in AD participants and correlated with t-tau, p-tau, and the four synaptic markers in pre-symptomatic individuals. In the latter, CSF apoB levels correlated with PET flortaucipir-binding in entorhinal, parahippocampal, and fusiform regions. Baseline CSF apoB levels were associated with longitudinal visuospatial cognitive decline.

Discussion: CSF apoB markedly associates with early tau dysregulation in asymptomatic subjects and identifies at-risk individuals predisposed to develop visuospatial cognitive decline over time.

KEYWORDS
Alzheimer's disease, apolipoprotein B, cerebrospinal fluid, PET scans, RBANS, synaptic markers, tau pathology

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1 | BACKGROUND

Elevated plasma cholesterol levels are among the established vascular risk factors of sporadic Alzheimer's disease (SAD),[1,2] whereas higher mid-life apolipoprotein B (apoB)–containing low-density lipoprotein (LDL) in blood has been associated with increased risk of developing SAD later in life, nominating LDL as an important discriminating factor in dementia etiology. In contrast, high cholesterol in late life does not appear to be associated with any form of dementia, or cognitive decline.[3,4] In recent months, two parallel lines of evidence have suggested that circulating LDL levels play an active role in the pathogenesis of early-onset familial Alzheimer's disease (EOAD). The association in EOAD was shown to be driven in large part by the presence of rare coding mutations in the *APOB* gene, suggesting a pathophysiological role for apoB-bound LDLs.[5]

At the genetic and molecular levels, several apolipoproteins have been directly implicated in the etiopathology of SAD, including *APOE*, clusterin (*CLU* or apoJ), and now *APOB*.[5–7] Apolipoproteins, such as apoB, apoE, and apoJ, as well as apoC3 and apoA1, combine to form soluble lipoproteins (such as high-density lipoprotein [HDL]), which serve as lipid transporters in the blood and CSF. Although there are no detectable levels of LDL in the CSF, significant amounts of apoB protein can be detected using sensitive magneto-fluorescent assays, whereas brain *APOB* messenger RNA (mRNA) is easily detected with RNA-Seq techniques.

Patients with SAD typically exhibit increased blood levels of apoB and LDL along with decreased HDL levels,[8,9] which correlate positively in post-mortem studies with brain tissue amyloid beta (Aβ)42 levels.[10] In transgenic mice, life-long *Apob* overexpression induces significant cognitive decline in the Morris water maze in mid-life, and is accompanied by apoB protein accumulation in cerebral vessels, combined with significant astrogliosis.[11] These observations prompted us to examine the neurobiology of the *APOB* gene and associated proteins in the brain of cognitively unimpaired "at-risk" subjects with a parental history of SAD (the PRe-symptomatic EValuation of Experimental or Novel Treatments for AD [PREVENT-AD][12] cohort), in cognitively unimpaired, mild cognitive impairment (MCI), and SAD subjects from the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort and, in the autopsied brains of cognitively unimpaired as well as persons with MCI and SAD (ROS-MAP cohort).

RESEARCH IN CONTEXT

1. Systematic review: Apolipoprotein B (apoB) was shown to be elevated in the plasma of subjects with Alzheimer's disease (AD) and mild cognitive impairment (MCI). However, levels of apoB have never been examined in the cerebrospinal fluid (CSF) in AD, or in relation to CSF tau alterations and deposition by positron emission tomography (PET) imaging.
2. Interpretation: Significant correlations were observed between CSF apoB and t-tau, P-tau, and four different synaptic markers in cognitively unaffected elderly patients with a parental history of AD. These changes correlated with the longitudinal decline of visuospatial cognitive performance over 6 to 8 years. ApoB acts an early biomarker of tau and synaptic pathology in AD and could be used in timing interventions in "at-risk" subjects.
3. Future directions: A longitudinal investigation is underway to compare cognitive and structural changes in cognitively unaffected subjects with high and low apoB. A clinical outcome of elevated apoB in relation to the apoliprotein E gene (*APOE*) ε4 allele should be investigated as a function of longitudinal amyloid alterations.

2 | METHODS

This study received local approval from the research ethics committees or institutional review boards of the participating centers.

2.1 | PREVENT-AD cohort

2.1.1 | Study participants

PREVENT-AD is an observational cohort of healthy older adults at increased risk of AD dementia.[12] PREVENT-AD enrolled more than 400 cognitively unimpaired participants age 60 years or older having a parent or at least two siblings diagnosed with AD dementia. Participants were followed-up annually with structural and functional magnetic resonance imaging (MRI), medical, and cognitive assessments. Participants also gave blood at each visit, and a subset of 160 volunteered for at least one lumbar puncture (LP). More recently, a partially overlapping sample (n = 129) also volunteered for brain positron emission tomography (PET) scans to assess Aβ and tau deposition.

2.1.2 | CSF measurements

Lumbar punctures were performed using a Sprotte 24-gauge atraumatic needle following an overnight fast. CSF samples were centrifuged within 4 hours to exclude cells and insoluble material. Blood samples are obtained before LPs to ensure a temporal relationship between peripheral and CNS measures. ApoB, apoC3, and apoE levels were measured using the apolipoprotein Luminex assay kit (10-plex magneto-fluorescent immunoassays, cat# 12003081, BioRad, USA). Because of sensitivity issues with apoB analyses, a second more sensitive Luminex milliplex-map assay was used for apoB in some subjects without dilution for the final determination (APOBMAG immunoassay from EDS-Millipore, Cat.# APOMAG-62K, Canada). The CSF AD biomarkers P-tau, t-tau, and Aβ42 were measured using a validated Innotest ELISA kits (P_{181}-tau Cat.# 81581, t-tau Cat.# 81579, and Aβ42 Cat.# 81583) from Fujirebio, Ghent, Belgium, following procedures from the biomarkers for Alzheimer's and Parkinson's disease (BIOMARKAPD) consortium.[13] Data were collected between

September 2011 and August 2017 and archived in PREVENT-AD data release 5.0 (https://openpreventad.loris.ca/). Immunoprecipitated synaptosome associated protein 25 (SNAP-25) and synaptotagmin from CSF were analyzed using high-resolution selected ion monitoring (HR-SIM) analyses on a quadrupole–orbitrap mass spectrometer Q Exactive as described in Brinkmalm et al.[14] and Ohrfelt et al.[15] CSF neurogranin and GAP-43 concentrations were assessed using validated enzyme-linked immunosorbent assays (ELISAs) described before.[16,17]

2.1.3 | PET image acquisition and processing

Aβ and tau pathologies were quantified using ^{18}F-NAV4694 (Navidea Biopharmaceuticals, Dublin, OH, USA) and flortaucipir (^{18}F-AV1451; Eli Lilly & Company, Indianapolis, IN, USA). Amyloid and tau scans were acquired 40 to 70 and 80 to 100 minutes post-injection. T1-weighted structural MRI scans were obtained using a 3T Siemens Trio scanner at the Douglas Mental Health Research Institute (Montreal). Aβ positivity was determined as described recently in McSweeney et al.[18] AD-related tau deposition was assessed by averaging flortaucipir standard uptake value ratio (SUVR) in the entorhinal cortex, fusiform, parahippocampal, and lingual giri.[18,19]

2.1.4 | Genotyping and imputation

Automated DNA extraction from buffy coat samples was performed using the QiaSymphony DNA mini kit (Qiagen, Toronto, Canada). Genotypes were determined with the Illumina Infinium Omni2.5 M-8 array (Illumina, San Diego, CA, USA). The PLINK tool set (http://pngu.mgh.harvard.edu/purcell/plink/) was used to (1) filter gender mismatches, (2) filter missingness at both the sample-level (<5%) and single-nucleotide polymorphism (SNP)-level (<5%), (3) assess sample heterozygosity, and (4) filter SNPs in Hardy-Weinberg disequilibrium ($P > .001$). Only post-imputed SNPs with an info score >0.7 were considered.

2.1.5 | Cognitive testing

Participants' cognitive performance was measured annually using the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS),[20] which evaluates five cognitive domains (immediate memory, delayed memory, attention, language, and visuospatial abilities) as well as a total summary score. The RBANS is available in four equivalent versions and was administered in French or English depending on the preferred language of the participants.

2.2 | COMPASS-ND cohort

2.2.1 | Study participants

This study is enrolling 1650 memory-impaired/concerned subjects from 31 centers across Canada. Participants typically undergo comprehensive baseline evaluation, including clinical and neuropsychological assessment, biospecimen collection, polymorphism mapping, and MRI neuroimaging.[21] Data are made available to investigators in the Canadian Consortium on Neurodegeneration in Aging (CCNA) as well as others through the Longitudinal Online Research and Imaging System (LORIS) database at https://ccna-ccnv.ca/national-platforms/. CSF collection and measurements are performed as described above for the PREVENT-AD Cohort.

2.3 | Alzheimer's Disease Neuroimaging Initiative (ADNI)

ADNI CSF and genetic data sets were downloaded from the ADNI website (www.loni.ucla.edu/ADNI).

2.3.1 | CSF measurements

The CSF multiplex multiple reaction monitoring (MRM) mass spectrometry panel consists of 567 peptides representing 221 proteins, and for each peptide two or more mass transitions were monitored. Two hundred ninety unique ADNI-1 baseline subjects are represented: 87 healthy control (CTL) subjects, as well as 66 with AD dementia and 136 with MCI. Three distinct peptides were quantified for apoB: TGISPLALIK, IAELSATAQEIIK, and SVSLPSLDPASAK. A thorough discussion of the methodology is available in Kennedy et al.[22] The Biomarker Consortium CSF Proteomics MRM consolidated dataset (CSFMRM.csv) is available directly from ADNI at http://adni.loni.usc.edu/.

2.4 | eQTL analyses

For quantitative traits analyses (PREVENT-AD and ROS-MAP data sets), regression statistics were calculated with PLINK v1.09.[23] The eQTL analysis was run in R (http://www.R-project.org) using the MatrixEQTL package.[24]

2.5 | Statistical analyses

We compared PREVENT-AD demographic characteristics of Aβ-negative and Aβ-positive, *APOE* ε4-negative and *APOE* ε4-positive, and tau-negative and tau-positive unimpaired older adults using Fisher exact or Kruskal-Wallis tests where appropriate (Table S1). We then tested for associations between CSF AD biomarkers (Aβ42, t-tau, P-tau) with CSF apolipoproteins (apoB, apoE, apoC3) using general linear models, adjusted for age and gender. We also tested for association between CSF apoB levels with global cortical NAV4694 SUVR and flortaucipir retention in the entorhinal cortex area and in the fusiform, parahippocampal, and lingual gyri using general linear models, controlling for age and sex. Similar models tested for associations of CSF apoB with plasma apoB, plasma to CSF albumin ratio, CSF microproteins

TABLE 1 PREVENT-AD cohort demographics

	Gender (Mean ± SEM)		ApoE Genotype (Mean ± SEM)		
	Female (n = 120)	Male (n = 49)	ApoE4− (n = 103)	ApoE4+ (n = 66)	E4+ vs E4- *P*
Age	62.10 ± 0.45	61.81 ± 0.73	62.64 ± 0.52	61.05 ± 0.52	
CSF Aβ42 (pg/mL)	1208.89 ± 30.98	1104.29 ± 44.32	1280.42 ± 29.08	1006 ± 38.70##	.0001**
CSF t-tau (pg/mL)	280.37 ± 14.56	289.28 ± 19.71	274.30 ± 14.85	296.48 ± 19.16	
CSF p-tau (pg/mL)	48.76 ± 1.90	49.87 ± 2.45	48.26 ± 1.96	50.36 ± 2.41	
CSF APOB (μg/mL)	0.80 ± 0.03	0.83 ± 0.04	0.70 ± 0.02	0.99 ± 0.03##	.0001**
CSF APOC3 (μg/mL)	0.048 ± 0.001	0.051 ± 0.003	0.049 ± 0.04	0.049 ± 0.02	
CSF APOE (μg/mL)	2.35 ± 0.11	2.61 ± 0.15	2.65 ± 0.12	2.42 ± 0.13	
CSF/Plasma albumin Ratio	0.0052 ± 0.0002	0.0064 ± 0.0004	0.0054 ± 0.0003	0.0057 ± 0.0004	
PET amyloid SUVR	1.31 ± 0.04	1.36 ± 0.06	1.28 ± 0.04	1.39 ± 0.05	
PET tau SUVR –Entorhinal Ctx	1.08 ± 0.02	1.08 ± 0.03	1.07 ± 0.03	1.10 ± 0.03	
MOCA	28.03 ± 0.16	27.55 ± 0.25	27.68 ± 0.17	28.28 ± 0.20#	.04*
RBANS (total score)	102.80 ± 1.03	97.6 ± 1.42	101.18 ± 1.02	101.13 ± 1.57	

Differences between E4− and E4+: #: $P < .05$ and ## $P < .01$.
*$p < 0.05$
**$p < 0.001$

content, and red blood cell density in the CSF as independent indexes of blood-brain barrier integrity and CSF tap blood contamination. Independent *t* tests were used for comparison of ROS-MAP *APOB* mRNA levels as a function of gender, *APOE* ε4 status, CERAD, and Braak stages.

3 | RESULTS

3.1 | Demographic characteristics

PREVENT-AD participants had a mean age of 63.95 ± 5.00 years at baseline and 68.50 ± 5.49 at PET assessment, and 85% were female. Additional demographic data are reported in Table 1. COMPASS-AD cohort subjects (n = 64) had a mean age at baseline of 62.43 ± 4.84, 70.78 ± 6.98, and 74.3 ± 6.92 for subjects with Parkinson, MCI, and sporadic AD, respectively, and 54% were female. ROS-MAP autopsy-confirmed subjects (cognitively unaffected, MCI, and AD) had a mean age at death of 83.57 ± 4.75 years, and 61% were female (see Supplementary material).

3.2 | CSF apoB level as a function of cognitive status

Figure 1 (top) illustrates the CSF apoB levels measured in cognitively unaffected (CTL) subjects, idiopathic Parkinson disease (IPD), mild cognitively impaired (MCI), and sporadic Alzheimer's disease (SAD) subjects from the COMPASS-AD cohort. CSF apoB levels in the SAD group were significantly higher than in CTL using one-way analysis of variance (ANOVA; $P = .009$). Figure 1 (bottom) shows results from a replication study in ADNI using MRM LC/MS-MS analysis of baseline apoB protein levels measured in the CSF of CTL, MCI, and SAD subjects. CSF apoB levels in SAD were found to be statistically different from control group using one-way ANOVA ($P = .05$).

3.3 | CSF apolipoproteins measures associate with CSF measures of total-tau and P-tau

Among all CSF biomarkers evaluated in PREVENT-AD, only CSF apoB and Aβ42 were shown to be affected by the presence of the *APOE* ε4 allele (Table 1). Baseline CSF apoB levels showed highly significant associations with t-tau and P-tau ($R^2 = 0.23$ and 0.28, both $P < .0001$, Figure 2). However, CSF apoB did not correlate with CSF Aβ42 ($R^2 = 0.003$, $P = .70$, Table S1).

Further stratifications of the relationships between apoB and t-tau or P-tau and Aβ42 by gender, *APOE* genotype, PET Aβ positivity, CSF t-tau positivity, and statin use are summarized in Table S1. The associations between CSF apoB and t-tau and P-tau held true for all stratifiers, except for PET Aβ positivity, where the association between apoB and t-tau was inapparent in Aβ-positive subjects. Of interest, none of the stratifiers affected the absence of association between apoB and CSF Aβ42 except for *APOE* genotype, where an association emerged as highly significant in *APOE* ε4-negative subjects only ($R^2 = 0.171$, $P < .001$, Table S1).

Analyses of cortical Aβ-PET binding failed to show any association with CSF apoB in PREVENT-AD subjects (Figure 3, top left). In contrast, Figure 3 shows significant associations between PET tau index and CSF apoB levels in the entorhinal cortex area (Braak stage I: R^2: 0.21, $P = .026$) and in the fusiform (R^2: 0.24, $P = .003$), parahippocampal (R^2: 0.17, $P = .006$), and lingual gyri (Braak stage III: R^2: 0.20, $P = .006$).

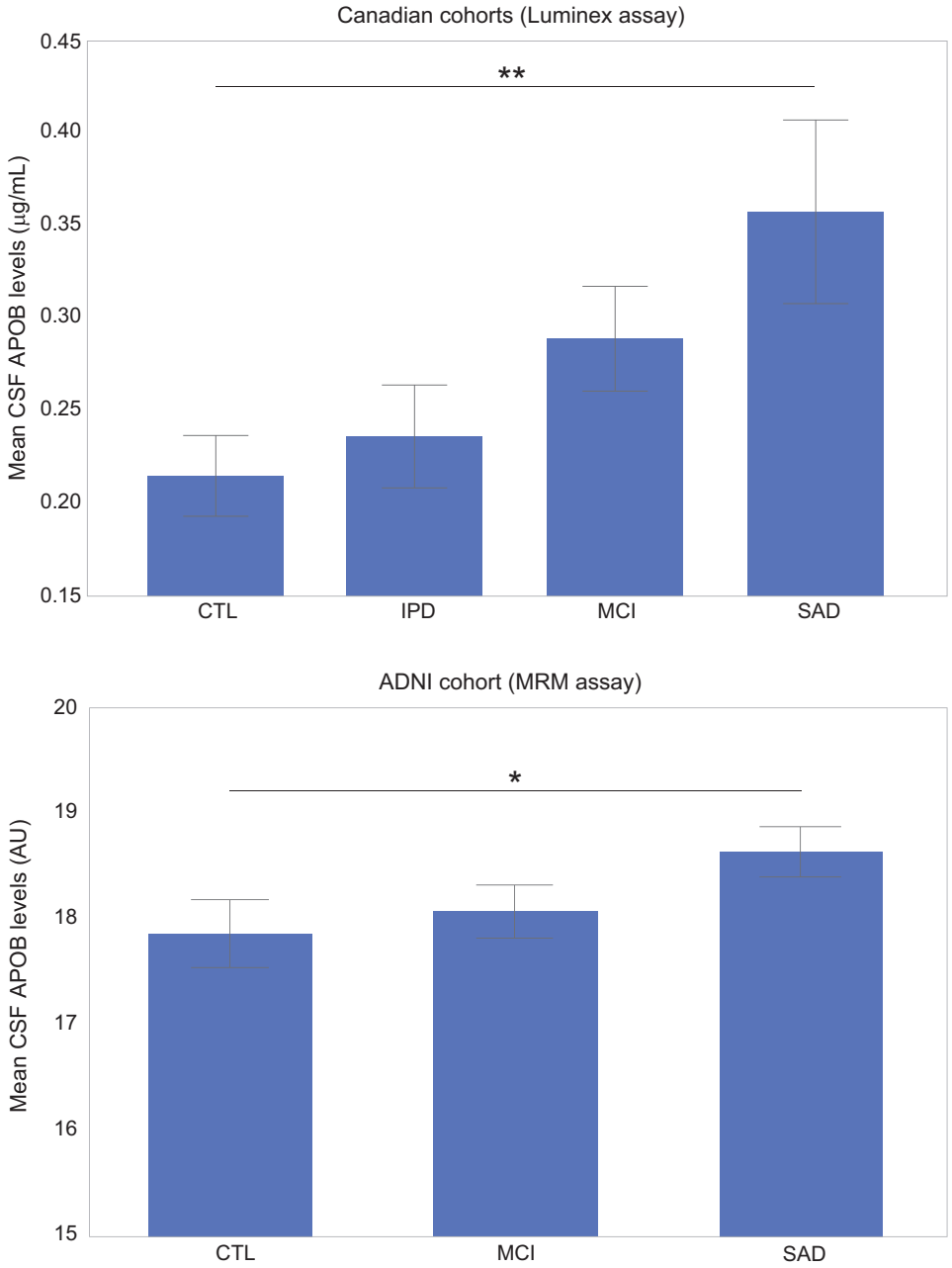

FIGURE 1 Cerebrospinal fluid (CSF) apolipoprotein B (ApoB) levels as a function of cognitive status. CSF ApoB levels were measured by Luminex assay for a subset of CSF samples (n = 64) from the Canadian cohorts (top) comprising cognitively unaffected individuals (CTL), memory-impaired/concerned subjects affected by idiopathic Parkinson disease (IPD), mild cognitive impairment (MCI), or sporadic Alzheimer's disease (SAD). In the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort (bottom), CSF ApoB levels were measured by MRM assay in 87 CTL, 136 MCI, and 66 SAD subjects. *P* values are indicated with asterisks: * $P = .05$, ** $P = .009$

3.4 | CSF apoB associates with visuospatial cognitive performance in unimpaired elderly

In linear regression analyses, baseline CSF apoB levels correlated with the visuospatial cognitive performance trajectory slopes estimated over the course of 6 to 8 years on the RBANS ($R^2 = 0.13$, $P < .02$, Figure 4). We found no significant interaction between CSF apoB and other subscales of the RBANS, or with the RBANS total score trajectory when adjusted for age, *APOE* ε4 status, education, and gender (Figure 4).

3.5 | CSF apoB associated with multiple synaptic markers in cognitively unimpaired elderly

Figure 5 illustrates the associations between apoB and neurogranin ($R^2 = 0.15$, $P < .01$), SNAP-25 ($R^2 = 0.19$, $P < .005$), synaptotagmin-1

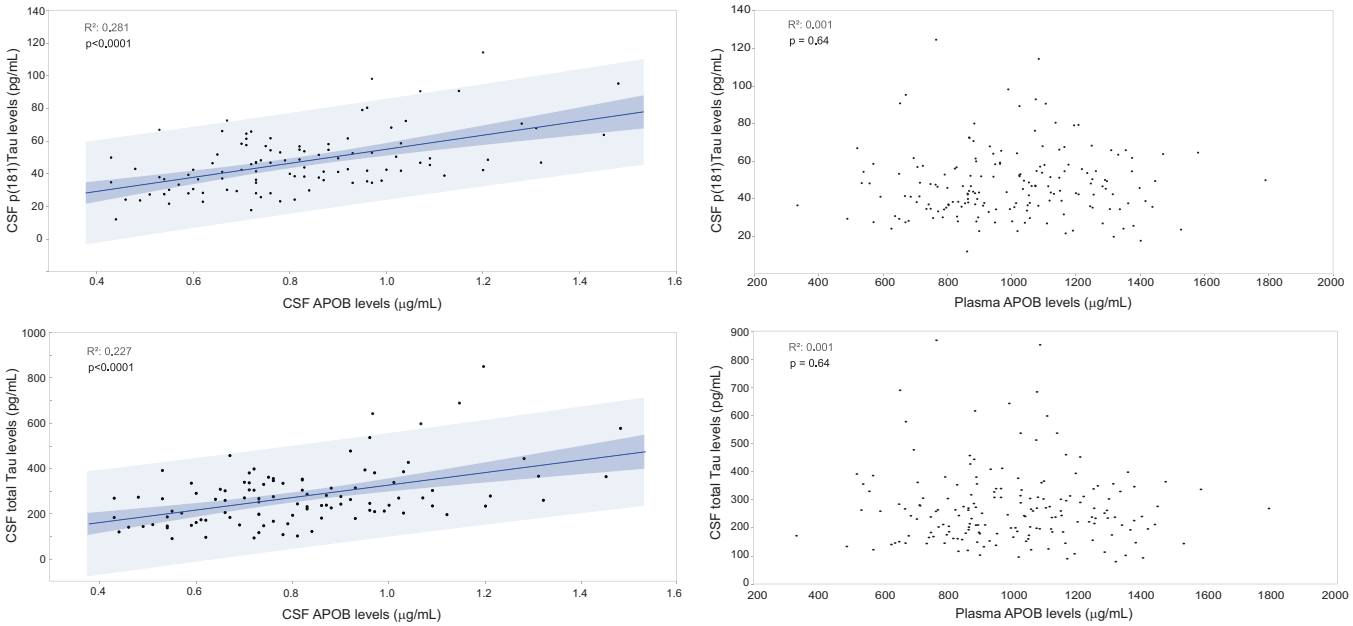

FIGURE 2 CSF and plasma apoB levels compared with CSF AD biomarkers p(181)-tau and t- tau in the PREVENT-AD cohort. CSF (left panels) and plasma (right panels) apoB levels were measured using the sensitive simplex APO-magnetic assay in 160 cognitively normal participants from the PREVENT-AD cohort. The CSF AD biomarkers p-tau (upper panels) and tau (lower panels) were measured by ELISA according to the procedures from the BIOMARKAPD consortium of the EU Joint Program in Neurodegenerative Diseases. Significant linear regressions are represented with a blue confidence region of the fitted line. Individual R squares and *P* values are shown in the top left corners of each figures

($R^2 = 0.18$, $P < .005$), and GAP-43 ($R^2 = 0.15$, $P < .01$) in the CSF of a subset of cognitively unaffected PREVENT-AD subjects at baseline.

3.6 | CSF apoB increases are not due to blood-brain barrier alterations or peripheral vascular burden

We measured the levels of albumin in both the plasma and CSF of PREVENT-AD subjects to establish an individual blood-brain barrier index. This was subsequently contrasted with CSF levels of apoB, apoE, and apoC3. Figure S1 illustrates results obtained for all three CSF apolipoproteins using the CSF-to-plasma albumin ratio to control for blood-brain barrier integrity. As expected, CSF apoB and apoE did not correlate with the albumin ratio in contrast to CSF apoC3, which is not produced in the central nervous system (CNS). We also contrasted CSF apoB levels to CSF microprotein level, white blood cell count, and red blood cell count in the CSF and found no associations (Figure S2). Finally, we examined the possible associations between CSF apoB, apoC3, and apoE and their plasma counterparts. We found weak associations between CSF and plasma apoC3 ($R^2 = 0.05$, $P = .05$) and apoE ($R^2 = 0.04$, $P = .04$), but none for apoB (Figure S3), further supporting that CSF apoB comes from the brain.

Using the Cardiovascular Risk Factors, Aging, and Incidence of Dementia (CAIDE) risk score, we examine the possible contribution of cardiovascular burden to the observed apoB behavior.[25] The CAIDE score, which includes age, hypertension, hypercholesterolemia, physical inactivity, obesity, *APOE* ε4, and educational level as model parameters, has been validated in several multi-ethnic populations in the United States and Europe. Table S2 summarizes the results obtained in the PREVENT-AD cohort. Except for the *APOE* ε4 CAIDE sub-score, all other sub-scores showed no apparent association with CSF apoB, apoC3, and apoE levels.

3.7 | Pan-genomic quantitative trait loci (QTL) analyses of brain *APOB* gene expression and CSF apoB protein levels identify distinct genomic regulators in cognitively unaffected individuals

Quantitative trait loci (QTL) analysis was performed in PREVENT-AD subjects to scan the genome for genetic polymorphisms affecting CSF apoB protein levels in these asymptomatic subjects. The single candidate gene that distinguished itself from all others was *APOE*, for which polymorphism at rs56131196 (-log(p) = 11.8) and rs429358 (-log(p) = 10.4) displayed genome-wide significant associations (Figure S4, top). Using the same strategy, we considered cognitively unaffected subjects from ROS-MAP autopsy-confirmed asymptomatic control subjects to scan the genome for associations with brain prevalence of *APOB* mRNA. Figure S4 (bottom) shows the Manhattan plot, which displays three interesting candidates: *YAE1* (rs4720330, -log(p) = 8.33), *RNASET1* (rs3778439, -log(p) = 7.88), and *PPARG* (rs2972165, -log(p) = 7.19). Note the absence of signal on chromosome 19 in the vicinity of the *APOE* locus.

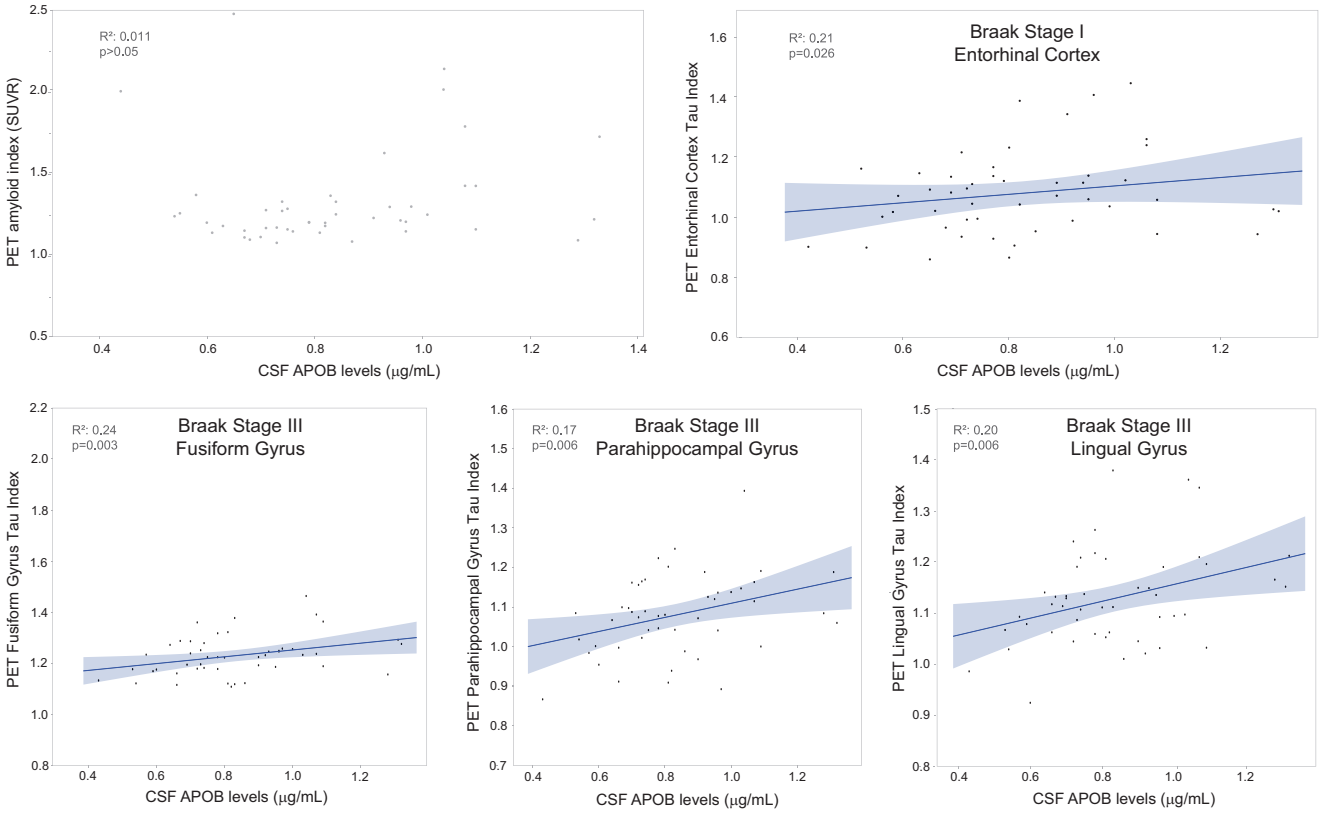

FIGURE 3 CSF apoB levels as a function of PET amyloid and tau index in the PREVENT-AD cohort. CSF apoB levels were measured using the sensitive simplex APO-magnetic assay and contrasted with PET scans measures of Aβ (18F-NAV4694: top left) and tau (Flortaucipir) in different brain sub-regions classified according to Braak stages (Braak stage I: entorhinal cortex, top right; Braak stage III: fusiform gyrus, bottom left; parahippocampal gyrus, bottom center; and lingual gyrus, bottom right). Significant linear regressions are represented with a blue confidence region of the fitted line. Individual *R* squares and *P* values are shown in the top left corners of each figures

3.8 | Apolipoprotein mRNAs do not associate with cortical tau and Aβ pathologies in cognitively affected subjects

Figure S5 illustrates cortical APOB mRNA prevalence across Braak (tangles) and CERAD (plaques) stages, stratified by *APOE* genotypes. Gene expression appears stable across the spectrum of tau and Aβ pathological changes.

4 | DISCUSSION

Current dogma on the presence of apoB in the CNS holds that, although detectable in the CSF, apoB is not produced there, but is likely instead caused by an influx from the periphery due to a porous blood-brain barrier.[26] The recent development of sensitive magneto-fluorescent assays for apoB and highly sensitive and specific RNA sequencing methods have helped elucidate the situation. Regional distribution of brain *APOB* mRNA by RNASeq in humans shows elevated expression in cerebral cortex, hippocampus, and medulla, with little or no signal in the olfactory region, amygdala, thalamus, and basal ganglia.[27] In situ hybridization shows similar regional distribution in the mouse CNS and microglial specificity.[28] A survey of the human and mouse brain transcriptome PanglaoDB databases using single cell RNA sequencing in the CNS indicates that *APOB* mRNAs are restricted to the microglia and astrocytic compartments in the hippocampus (https://panglaodb.se/view_interactive_tsne_data.html?sra=SRA675945&srs=SRS3100152&plot=tsne&overlay=APOB). These recent findings support the notion that apoB is synthesized locally in the brain, secreted extracellularly, and detected in the CSF where it is most likely used in the maintenance of lipid homeostatic processes.

Findings of strong associations between CSF apoB, t-tau, and P-tau (but not Aβ42, Figure 2) in "at-risk" subjects are novel but consistent with genome-wide association studies (GWAS) that have demonstrated strong associations between SAD risk and common polymorphisms in genes directly involved in brain cholesterol metabolism, including *APOE*, *ABCA1*, *ABCA7*, *ABCG1*, *BIN1*, *PICALM*, *CLU*, and *SORL1*.[7,29–32] The absence of association between plasma apoB, t-tau, and P-tau emphasizes the specific relevance of this observation to the CNS.

Last year a meta-analysis of three different cohorts of early-onset AD subjects[5] found a strong association between rare genetic coding variants of *APOB* and the familial form, independent of *APOE* ε4 allele,[5] adding to the existing transgenic mice literature which shows that

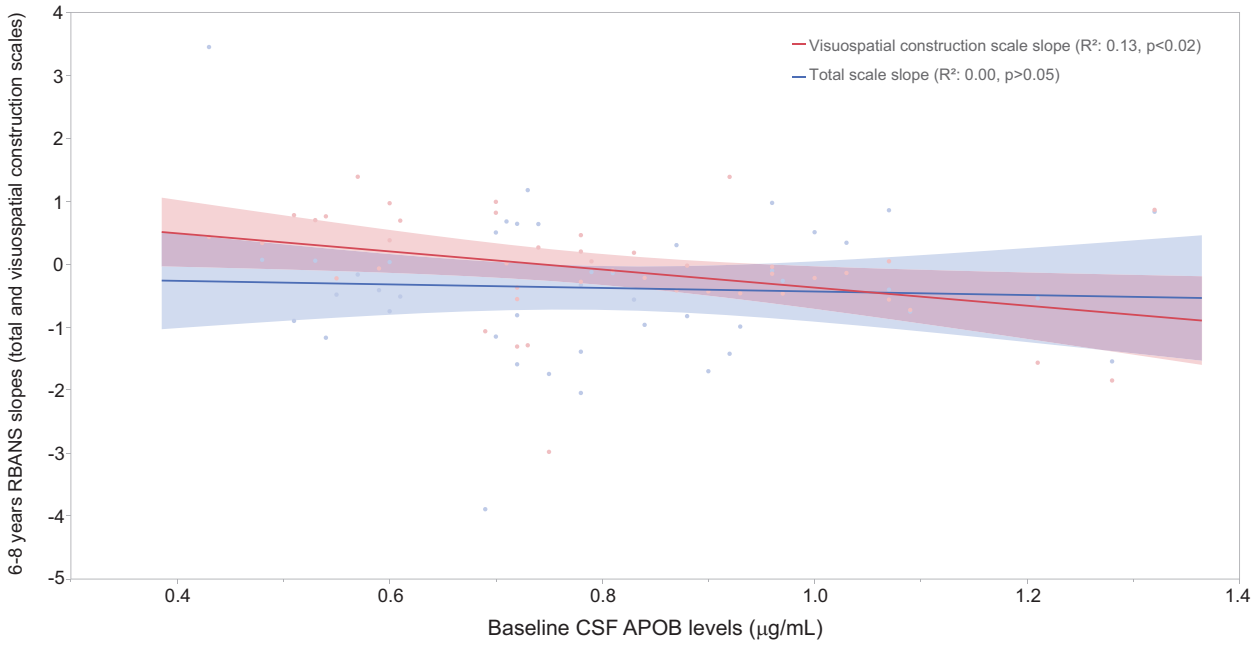

FIGURE 4 CSF apoB levels as a function of cognitive performance in the PREVENT-AD cohort. CSF apoB levels were measured using the sensitive simplex APO-magnetic assay and contrasted with cognitive performance assessed over a period of 8 years using the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS). Linear regressions are represented with a confidence region of the fitted line (red for visuospatial construction scale and blue for total scale)

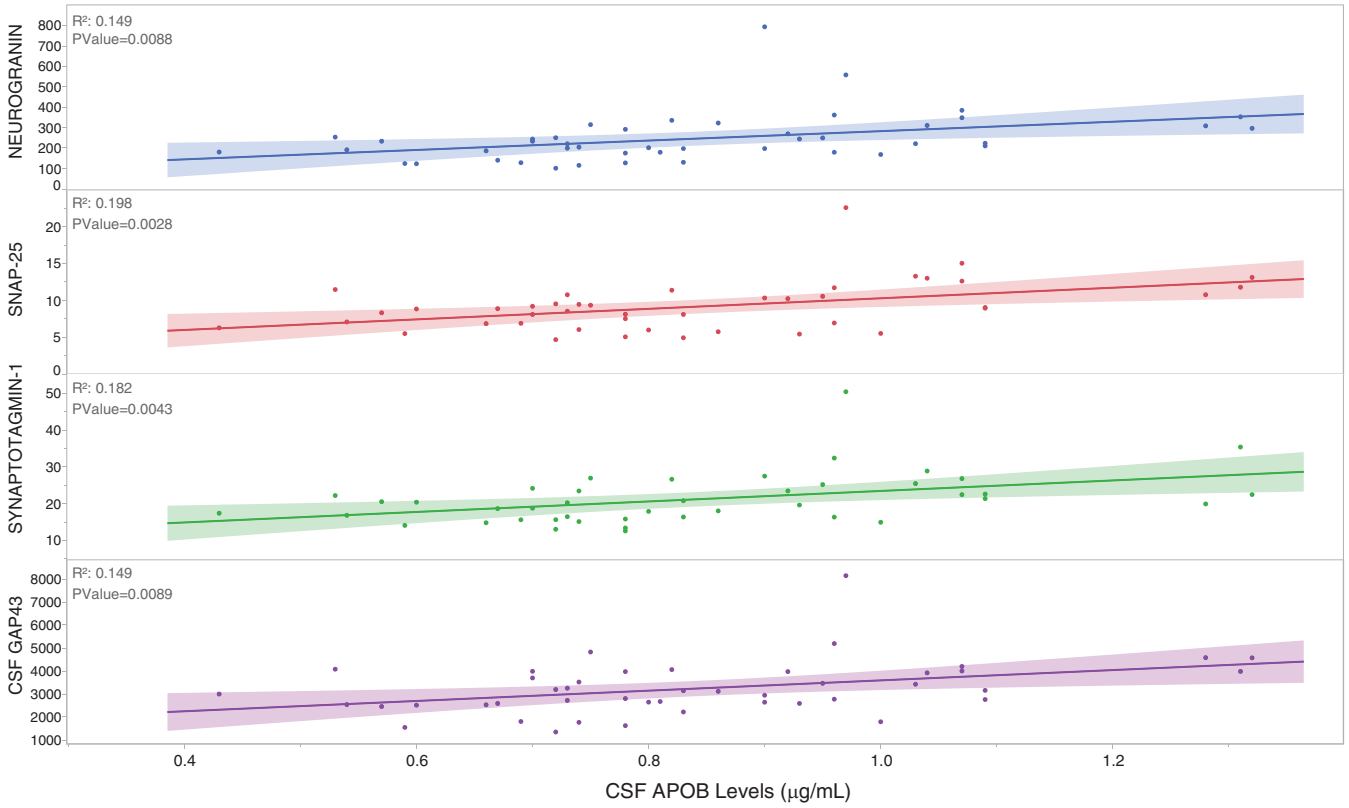

FIGURE 5 CSF apoB levels contrasted with synaptic markers in the PREVENT-AD cohort. CSF apoB levels were measured using the sensitive simplex APO-magnetic assay, and the synaptic markers were quantified using SRM mass spectroscopy. Significant linear regressions are represented with a colored confidence region of the fitted line. Individual *R* squares and *P* values are shown in the top left corners of each figure

life-long exposure to high apoB levels in *Apob* transgenic animals leads to significant neurodegenerative changes in the brain,[33] hyperphosphorylation of tau protein in the absence of amyloid deposition, extensive cortical and hippocampal neuronal apoptosis, marked reduction in the number and size of the dendritic spines in the hippocampal neurons, and impaired hippocampal presynaptic function.[34] Aging combined with *Apob* overexpression induces significant cognitive decline in the Morris water maze at mid-life.[11] However, in contrast to the increased levels of cholesterol in plasma, no substantial changes were detected in the cerebral cholesterol level of aging *apoB* transgenics compared to wild-type littermates, suggesting a cholesterol-independent pathological mechanism in the brain.[34]

In the cognitively unaffected pre-symptomatic PREVENT-AD subjects, the observed associations between CSF apoB and t-tau and P-tau is not associated with cerebral deposition of Aβ when using cerebral Aβ-PET binding but correlate with Flortaucipir binding in several brain regions known to be affected early by tau pathology in Braak stages 1 to III (Figure 3). It suggests that the role of apoB in early tau pathophysiology precedes amyloid deposition by several months or years. These results are consistent with cognitive evaluations performed longitudinally in a subset of PREVENT-AD participants; the trajectory (slope decline) of visuospatial constructional RBANS scores over 6 to 8 years correlates inversely with baseline CSF apoB level (Figure 4, R^2: 0.13, $P < .02$).

In order to evaluate the issue of peripheral vascular burden contribution, we systematically examined the associations between CSF apoB, apoC3, and apoE and the six sub-scores of the CAIDE index in PREVENT-AD. As expected, the *APOE* ε4-CAIDE subscore was found to significantly affect CSF apoB concentrations. However, none of the other sub-scores (body-mass index [BMI], circulating cholesterol levels, systolic blood pressure, education, and physical activity indices) showed association with CSF apoB levels (Table S2), suggesting a limited involvement of cardiovascular risk factors in the APOB/tau connection in the pre-symptomatic stage of the disease. However, this situation is bound to change over time as subjects' AD pathology progresses and symptoms emerge, something in conjunction with observable vascular changes.

Blood-brain barrier integrity is known to become progressively compromised in late MCI and AD subjects.[35] To examine this issue in our cognitively unaffected subjects, we performed several independent standard analyses. The ratio of CSF to blood albumin failed to detect any association with CSF apoB as opposed to apoC3, which originates almost exclusively from blood (Figure S1, bottom graph). In addition, we contrasted CSF apoB levels with the microprotein content, red blood cell, and white blood cell counts in the CSF (Figure S2) and found no association. Finally, we contrasted CSF and plasma levels of each of our target apolipoproteins and found no association for apoB, and weak associations for apoE ($R^2 = 0.04$, $P < .05$) and apoC3 ($R^2 = 0.05$, $P < .05$). Altogether, these results indicate that the presence of apoB in the CSF is not result of blood-brain barrier leakage of blood-derived apolipoproteins.

Taking advantage of the microarray data set from the ROS-MAP cohort, we examined whether brain *APOB* mRNA prevalence could explain the increased in CSF apoB observed with the emergence of cognitive deficits, especially as a function of CERAD and Braak stages. *APOB* gene expression remains stable throughout the course of AD in the pre-frontal cortex, also unaffected by the presence of the *APOE* ε4 allele (Figure S5). This unexpected finding in the frontal cortex led us to contrast *APOB* gene expression in the brain (in cognitively unaffected ROS-MAP subjects) and CSF apoB protein levels (in cognitively unaffected PREVENT-AD subjects) with genomic data using QTL analysis. As shown in Figure S4, very different candidate genes emerged from the analyses. Only *APOE* reached genome-wide significance to explain CSF apoB increases. In contrast, *APOE* variants do not act as direct modulators of *APOB* gene expression in the cortex of asymptomatic subjects in ROS-MAP (Figure S4, bottom graph). Instead, three different genes reached the proper threshold: *RNASET1*, *YAE1*, and *PPARG*. When combined with the demographic characteristic (Table 1), these results indicate that *APOE* ε4 allele acts a prime regulator of apoB protein levels in the CSF but not of cortical *APOB* gene expression. It is conceivable that the absence of alteration in *APOB* gene expression in the frontal cortex in AD is due to regional differences and weaker than expected gliosis in this brain region. Additional studies are planned using the Harvard and Mayo brain bank RNASeq data sets (NCBI #GSE33000 and Synapse ID# syn5550404) to examine the regional specificity of *APOB* expression in presence and absence of AD, and other tauopathies.

So, if gene expression does not explain the observed *tau*-dependent apoB increase in the CSF, it is conceivable that reduced apoB protein degradation may be at play at this stage of the disease. We recently reported elevated proprotein convertase subtilisin/kexin type 9 (PCSK9) levels in cortical areas in autopsied AD and strong correlations between PCSK9 and apoB, as well as with P-tau in the CSF in PREVENT-AD subjects. PCSK9, which normally enhances LDLR catabolism and reduces apoB binding and internalization, could lead to the observed CSF apoB increase. This model is further supported by recent evidence from the Swedish bioFINDER study showing significant reduction of soluble LDLR protein levels in the CSF of AD subjects versus Aβ-negative controls,[36] an observation that we recently replicated in the CCNA cohort where both MCI and AD subjects display lower levels of sLDLR when compared to cognitively unaffected subjects (not shown).

The latter observations are especially meaningful in the context of compensatory synaptic remodeling in the adult brain, as apoE, apoD, apoJ, LDLR, and PCSK9[37–41] were all shown to regulate the brain response to synaptic loss by facilitating the transfer and mobilization of key lipids such as cholesterol and phospholipids from dead or dying neurons to healthy neurons actively engaged in synaptic turnover and replacement. As illustrated in Figure 6, the HDL-mediated lipid transport is central for the proper delivery of cholesterol and associated lipids involved in synaptic and terminal reconstruction. Apolipoproteins such as apoD, apoE, and apoJ were shown to facilitate the binding of HDL particles to cell-surface receptors belonging to the LDLR family.[42]

In this very specific context, we decided to explore the role of synaptic proteins in the CSF as an index of synaptic integrity and their

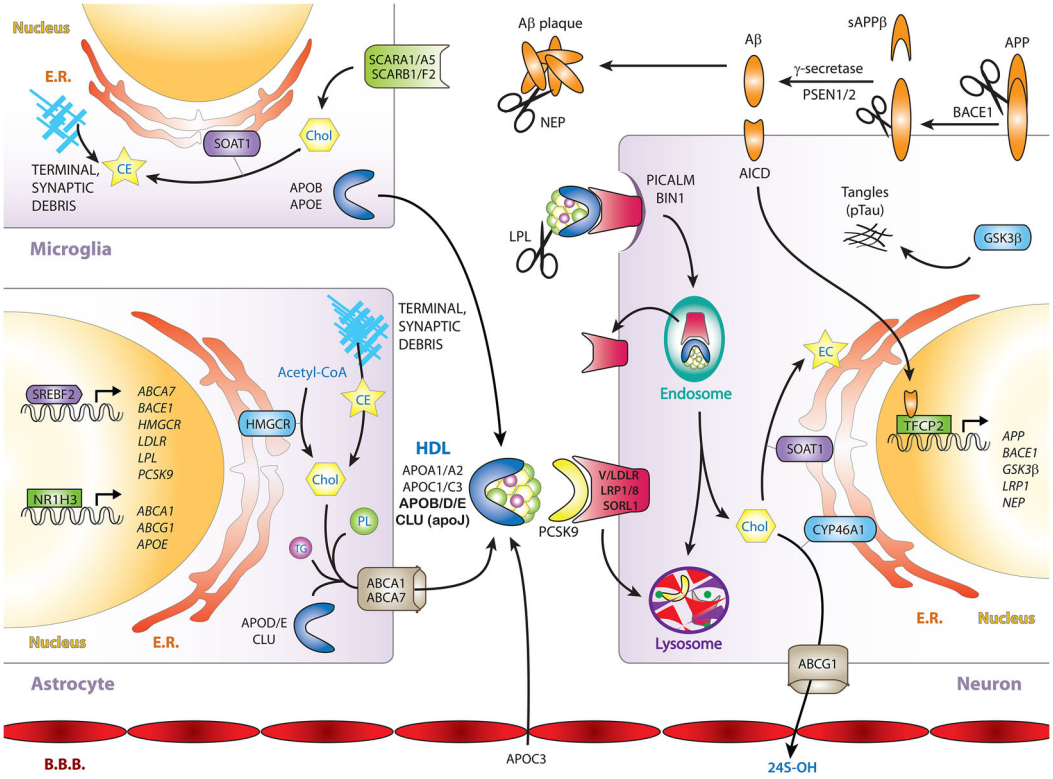

FIGURE 6 Apolipoproteins and cholesterol metabolism under neurodegenerative conditions. Genes (italicized) and gene products are identified in black, whereas other molecules or cellular compartment are depicted in colors. Abbreviations: 24S-OH, 24S-hydroxycholesterol; A*β*, amyloid beta, ABCA1/7, ATP binding cassette subfamily A member 1/7; AICD, amyloid precursor protein intracellular domain; ABCG1, ATP binding cassette subfamily G member 1; Acetyl-CoA, acetyl coenzyme A; APOA1/A2/B/C1/C3D/E/J, apolipoprotein A1/A2/B/C1/C3D/E/J; APP, amyloid beta precursor protein; BACE1, beta-secretase 1; B.B.B., blood-brain barrier; BIN1, bridging integrator 1; Chol, cholesterol; CLU, clusterin (alias APOJ); CYP46A1, cytochrome P450 family 46 subfamily A member 1; CE, cholesteryl ester; E.R., endoplasmic reticulum; GSK3*β*, glycogen synthase kinase 3 beta; HDL, high-density lipoprotein; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; LDLR, low-density lipoprotein receptor; LPL, lipoprotein lipase; LRP1/8, low-density lipoprotein receptor–related protein 1/8 (LRP8 alias APOER2); NEP, neprilysin; NR1H3, nuclear receptor subfamily 1, group H, member 3 (alias LXR); PCSK9, proprotein convertase subtilisin/kexin type 9; PICALM, phosphatidylinositol binding clathrin assembly protein; PL, phospholipids; PSEN1/2, presenilin 1/2; SCARA1/A5/B1/F2, scavenger receptor A1/A5/B1/F2; SOAT1, sterol O-acyltransferase 1 (alias ACAT); SORL1, sortilin-related receptor 1; SREBF2, sterol regulatory element binding transcription factor 2; TFCP2, transcription factor CP2 (alias LSF); TG, triglyceride; VLDLR, very low-density lipoprotein receptor

interactions with brain apolipoproteins at different stages of tau pathology. It is interesting to note that most CSF biomarkers that strongly associate with CSF t-tau and P-tau, but not with A*β*42, are synaptic proteins. These include both the dendritic protein neurogranin[43] and the pre-synaptic proteins SNAP-25[14] and synaptotagmin-1(15). In line with the above mechanistic model (Figure 6) and the known synaptotoxic properties of tau, we examined the possible relationship between apoB, tau, and four key synaptic proteins found in the CSF. Figure 5 illustrates the significant associations found between CSF apoB levels and these four pre- and post-synaptic markers in a subset of cognitively unaffected PREVENT-AD subjects.

Neuronal synapse formation and remodeling is essential to CNS development and can become dysfunctional in age-related neurodegenerative diseases. Disruption of mechanisms controlling neuronal plasticity and remodeling, eventually resulting in a net loss of synapses, is clearly implicated the early pathological stages in AD.[44,45] Alterations in synaptic integrity and density occur before overt neurodegeneration and should not be considered to uniformly decrease over the course of the disease process. It is well known that synaptic levels are influenced by an interplay between processes of neurodegeneration and deafferentation, and those involved in the maintenance and compensatory response at regional and network levels. After neuronal damage occurs, neuronal circuits and the local environment are disrupted causing the accumulation of debris in the affected region. The rapid engulfment and clearance of such dead cells or debris is essential for the remodeling of the neuronal circuits and/or microenvironment. Until recently, the engulfment has been thought to be limited to professional phagocytes, that is, microglia in the brain. However, astrocytes were shown to actively contribute to the synapse elimination that mediate neural circuit refinement in the developing CNS by phagocytosing synapses and they continue to engulf intact and compromised synapses in the adult and aging CNS.[46,47] Astrocytes thus share with microglia the ability to actively engulf and eliminate synapses in response to impaired neural activity as well as degeneration, but synapse engulfment by astrocytes is independent of complement proteins[46,48] and uses distinct phagocytic pathways from

microglia. Microglia, on the other hand, move actively toward the site of damage, including ischemic, excitotoxic, and neurodegenerative insults, and engulf and eliminate neuronal debris after cell death.[3,5–7]

As illustrated in Figure 6, the important lipid-associated players that are actively involved in this process involve several members of the apolipoprotein family. Apolipoproteins such as apoE, apoB, and apoJ have been shown to facilitate extracellular cholesterol and phospholipid mobilization and transport via the HDL lipoprotein system in the CNS.[49,50] ABCA1, ABCG1, and ABCA7 coordinate the secretion of cholesterol from phagocytized neuronal debris[47,51] by astrocytes (mostly synaptic membrane) and microglia (terminals and dendrites). The resulting extracellular cholesterol-enriched HDLs can either escape the CNS or target neurons undergoing synaptic remodeling and terminal proliferation by providing the much-required cholesterol and phospholipids building blocks. Of interest, one of the HDL surface receptors in neurons is SORL1. It has been shown to be genetically associated by GWAS with sporadic AD, as is the case for APOE, CLU (apoJ), ABCA1, ABCG1, and ABCA7 risk genes.[32] These genetic findings when combined with the observed changes in multiple apolipoproteins in response to tau-mediated synaptic damage suggest a direct involvement of this molecular cascade in the pre-symptomatic phase of the disease, when glial-driven synaptic remodeling is activated in response to early synaptic damage. Furthermore, it has been demonstrated that this remodeling process is clearly compromised at the electron-microscopy level in carriers of the $\varepsilon 4$ allele in multiple brain regions in AD.[52,53] Because the brain is poorly equipped to store important quantity of membrane-derived lipids, the glial production of apoE, apoJ, and especially apoB is increased to facilitate the assembly of HDL particles, which will in turn transport the excess of lipids to the periphery via the blood-brain barrier or to nearby neurons undergoing synaptic and terminal remodeling. In this model, the presence of mutations and/or polymorphism lipid-associated genes that are genetically linked to AD may compromise the delicate equilibrium normally exists between synaptic loss and compensatory remodeling in the aging brain.

Figure 6 illustrates a most likely scenario by which CSF apoB originating from microglia contributes to this molecular cascade during the early phase of the disease process, when tau become phosphorylated at multiple sites, and P-tau and t-tau are released in the extracellular space. These extracellular alterations most likely signal to nearby by glial cells the activation of microglial (and astrocytic) phagocytosis to eliminate degenerating terminals and synaptic debris. The resulting accumulation of membrane-derived cholesterol in glial cells stimulates de novo synthesis and release of apoB, apoE, and apoJ to metabolically repackage the water-insoluble cholesterol into a functional HDL complex, which can then transport the recycled cholesterol and phospholipids to nearby reinnervating neurons or to escape the CNS via the blood-brain barrier.

In turn, local microglia are activated and apoB/apoE are synthesized and released, while in parallel, astrocytosis induce apoD/apoE/apoJ synthesis and secretion, all of which play an active role in the mobilization of lipids derived from damaged synapses and compromised terminals[37] prior to the activation of compensatory synaptic remodeling. In this scenario, all four apolipoproteins work in partnership with HDLs to mobilize and redistribute the membrane-derived cholesterol to either nearby reinnervating neurons or to the blood-brain barrier, where it is actively transferred to the blood stream for disposal, like any other lipoproteins (Figure 6).

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CONFLICTS OF INTEREST

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AUTHORS CONTRIBUTIONS

JP, CP, NN, JB, SV, JCB, KB, and HZ conceptualized the research. AL, NJA, HZ, and KB performed CSF and plasma biomarker measurements, data quality control, and data compilation. DA and CP performed the pan-genomic analysis of DNA samples and quantitative trait analyses. JP, CP, NN, AL, and SV contributed to data analysis. JP, SV, CP, and NN developed the algorithms for data analysis. JP, CP, NN, and SV wrote the original manuscript draft. All authors reviewed, edited, and approved the final manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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