

FEATURED ARTICLE

Neuropathology-based APOE genetic risk score better quantifies Alzheimer's risk

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Abstract

Introduction: Apolipoprotein E (APOE) $\epsilon 4$ -carrier status or $\epsilon 4$ allele count are included in analyses to account for the APOE genetic effect on Alzheimer's disease (AD); however, this does not account for protective effects of APOE $\epsilon 2$ or heterogeneous effect of $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$ haplotypes.

Methods: We leveraged results from an autopsy-confirmed AD study to generate a weighted risk score for APOE (APOE-npscore). We regressed cerebrospinal fluid

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(CSF) amyloid and tau biomarkers on *APOE* variables from the Wisconsin Registry for Alzheimer's Prevention (WRAP), Wisconsin Alzheimer's Disease Research Center (WADRC), and Alzheimer's Disease Neuroimaging Initiative (ADNI).

Results: The *APOE*-npscore explained more variance and provided a better model fit for all three CSF measures than *APOE* $\epsilon 4$ -carrier status and $\epsilon 4$ allele count. These findings were replicated in ADNI and observed in subsets of cognitively unimpaired (CU) participants.

Discussion: The *APOE*-npscore reflects the genetic effect on neuropathology and provides an improved method to account for *APOE* in AD-related analyses.

KEYWORDS

Alzheimer's disease, amyloid beta, *APOE*, biomarkers, cerebrospinal fluid endophenotypes, phosphorylated tau

1 | INTRODUCTION

The apolipoprotein E gene (*APOE*) is the predominant genetic risk factor for late-onset Alzheimer's disease (AD), with three alleles contributing to disease risk: $\epsilon 2$ (reduced risk), $\epsilon 3$ (reference), and $\epsilon 4$ (increased risk). *APOE* genotype is associated with many AD endophenotypes, biomarkers reflecting the underlying neuropathology of amyloid plaques and neurofibrillary tangles, such as cerebrospinal fluid (CSF)¹ and positron emission tomography (PET) measures of amyloid and tau.² The importance of accounting for the strong genetic effect of *APOE* on AD risk has been recognized in many analyses of AD-related outcomes and researchers often use *APOE* $\epsilon 4$ -carrier status (*APOE4*-status: $\epsilon 4+/\epsilon 4-$)^{2,3} or, less frequently, the number of *APOE* $\epsilon 4$ alleles ($\epsilon 4$ -count: 0, 1, 2).⁴ Using these methods to model *APOE* genetic risk has limitations: (1) *APOE4*-status and the $\epsilon 4$ -count do not account for the effects of reduced risk conferred by *APOE* $\epsilon 2$; (2) an assumption

of the allele count approach is that genetic risk of *APOE* $\epsilon 4$ is strictly additive; and (3) as a dichotomous variable *APOE4*-status has limitations in statistical modeling such as loss of statistical power or problems with model convergence.⁵ To overcome these limitations, we previously used a weighted score for *APOE* genotype based on risk for AD diagnosis.^{1,6} Another group used a similar method to model the *APOE* genetic effect in polygenic risk scores, weighting the number of *APOE* $\epsilon 2$ alleles and the number of *APOE* $\epsilon 4$ alleles by the effect sizes reported in the Kunkle et al. genome-wide association study⁷ for single nucleotide polymorphisms (SNPs) rs7412 (encoding $\epsilon 2$) and rs429358 (encoding $\epsilon 4$).⁸ One limitation of these *APOE* risk scores is that they are based on clinical diagnosis of AD dementia which can include preclinical AD appearing as controls and dementia cases due to non-AD causes.⁹⁻¹¹ Here, we propose an improved method to account for *APOE* genetic risk for AD in statistical analyses using a weighted score based on AD neuropathology, providing a pseudo-continuous

variable that does not collapse important genotype categories. By comparing AD cases and controls that were confirmed at autopsy, Reiman et al. showed that the odds ratio (OR) has been overestimated for *APOE* $\epsilon 2/\epsilon 2$ individuals and underestimated for *APOE* $\epsilon 4/\epsilon 4$ individuals in clinical risk studies.¹² Similar results have been obtained using CSF endophenotypes as surrogate measures of AD pathology.¹³ We propose that using this *APOE* neuropathology-based score (*APOE*-npscore) will help researchers more accurately account for the genetic effect of *APOE* on the underlying neuropathology of AD. This can increase statistical power, avoid modeling issues that result from low-frequency genotypes with low counts, and allows for a more nuanced variable that may help distinguish AD from diseases with similar clinical appearance. Furthermore, most studies can immediately incorporate the *APOE*-npscore in analyses because it is easily derived from existing *APOE* genotype data ($\epsilon 2$, $\epsilon 3$, $\epsilon 4$).

CSF biomarkers for amyloid-beta1-42 ($A\beta 42$) and amyloid-beta1-42/1-40 ($A\beta 42/40$) ratio and phosphorylated tau 181 (ptau181) are among the gold standard biomarkers for AD. CSF $A\beta 42$ and the $A\beta 42/40$ ratio decrease early in AD and are negatively correlated with amyloid PET¹⁴ and amyloid plaques presence, a hallmark neuropathology of AD.¹⁵ CSF $A\beta 42/40$ ratio has been reported to predict PET amyloid-positivity more accurately than CSF $A\beta 42$ alone, regardless of clinical diagnosis.^{16,17} Another key pathological hallmark of AD, neurofibrillary tangles composed of hyperphosphorylated tau, is positively correlated with CSF ptau181 concentration.¹⁸ The ratio of CSF ptau181/ $A\beta 42$ is predictive of cognitive decline and conversion to AD dementia.^{19–21} These CSF biomarkers change before cognitive symptoms appear^{19,22} and can help distinguish AD from other diseases that are clinically similar.^{23–25} The correlation with AD neuropathology and measurable changes early in disease made these CSF biomarkers ideal for testing our hypothesis that the *APOE*-npscore is an improvement over other methods in accounting for the *APOE* genetic risk for AD in statistical analyses.

2 | METHODS

2.1 | Participants

The Institutional Review Boards of all participating institutions approved the study, and research was carried out in accordance with approved protocols. Written informed consent was obtained from participants or their family members. Data were obtained from longitudinal studies of preclinical and clinical AD from the Wisconsin Registry for Alzheimer's Prevention (WRAP)²⁶ and the Wisconsin Alzheimer's Disease Research Center (WADRC). WRAP is a longitudinal observational cohort study, established in 2001, of middle-aged participants that is enriched with people who have a parental history of probable-AD dementia. The WADRC was established in 2009 and is one of the National Institute on Aging (NIA) –designated ADCs across the United States. Participants enrolled in these studies provided CSF within 1 year of cognitive testing. Diagnoses were determined by consensus conference of dementia specialists based

RESEARCH IN CONTEXT

- 1. Systematic Review:** We reviewed existing literature for current methods used to account for *APOE* genetic risk in Alzheimer's disease (AD) research. Although some studies used variables other than dichotomous *APOE* $\epsilon 4$ -carrier status (*APOE4*-status), none used weighted scores based on autopsy-confirmed AD. There have been no direct comparisons of different variables used to account for *APOE* genetic risk.
- 2. Interpretation:** We observed the neuropathology-based weighted *APOE* risk score (*APOE*-npscore) consistently provided better model fit for cerebrospinal fluid (CSF) AD endophenotypes than either *APOE4*-status or $\epsilon 4$ -count. Our findings demonstrate the benefit of using a pseudo-continuous variable like the *APOE*-npscore, which increases statistical power and avoids modeling issues from small sample sizes.
- 3. Future Directions:** The *APOE*-npscore can easily be implemented by studies with *APOE* genetic data for their participants. Additional studies in diverse cohorts are necessary to create a more refined *APOE*-npscore to account for *APOE* genetic risk in a broader context.

on NIA-Alzheimer's Association (NIA-AA) criteria without reference to biomarker status.^{27,28} The combined data in these analyses include participants with mild cognitive impairment (MCI), dementia due to suspected AD (dementia-AD), or cognitively unimpaired (CU) individuals.

Data used for replication analyses were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu).²⁹ The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), PET, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. For up-to-date information, see www.adni-info.org.

2.2 | Genotyping and scoring

DNA extracted from whole blood samples from WADRC and WRAP participants was genotyped for *APOE* $\epsilon 2$ and *APOE* $\epsilon 4$ using competitive allele-specific PCR-based KASP genotyping for rs7412 and rs429358, respectively.⁶ Data downloaded from the ADNI database were obtained from DNA extracted from blood, as described previously.³⁰

To generate the *APOE*-npscore we used a natural log (ln) transformation of the OR values reported in a study of *APOE* genetic risk in autopsy-confirmed AD cases ($n = 4018$) and controls ($n = 989$), none

of whom were participants in our analyses.¹² OR values were obtained from the Reiman et al., supplementary table which had OR calculated for each APOE genotype, using $\epsilon 3\epsilon 3$ as the reference, after adjusting for age and sex: $\epsilon 2\epsilon 2$ OR = 0.16, $\epsilon 2\epsilon 3$ OR = 0.40, $\epsilon 3\epsilon 3$ OR = 1, $\epsilon 2\epsilon 4$ OR = 2.47, $\epsilon 3\epsilon 4$ OR = 5.71, and $\epsilon 4\epsilon 4$ OR = 26.93¹². By using the $\ln(\text{OR})$, the APOE-npscore is negative for haplotypes associated with reduced risk for AD compared to $\epsilon 3\epsilon 3$, resulting in the APOE-npscore: $\epsilon 2\epsilon 2 = -1.833$, $\epsilon 2\epsilon 3 = -0.916$, $\epsilon 3\epsilon 3 = 0$, $\epsilon 2\epsilon 4 = 0.904$, $\epsilon 3\epsilon 4 = 1.742$, and $\epsilon 4\epsilon 4 = 3.293$.

2.3 | CSF collection and assays

CSF samples from WADRC and WRAP were acquired as described previously.³¹ Briefly, samples were collected by lumbar puncture (LP) in the morning after an 8- to 12-h fast, centrifuged to remove red blood cells or other debris, then 0.5 mL CSF was aliquoted into 1.5-mL polypropylene tubes and stored at -80°C within 30 min of collection. WRAP and WADRC CSF samples were assayed at the Clinical Neurochemistry Laboratory, University of Gothenburg under strict quality control procedures. A β 42, ptau181, and A β 40 levels in CSF were measured using the Elecsys β -Amyloid(1-42) CSF, Elecsys Phospho-Tau (181P) CSF, and Elecsys β -Amyloid(1-40) electrochemiluminescence immunoassays, respectively, on the cobas e 601 analyzer (all Roche Diagnostics International Ltd.).

ADNI CSF samples, obtained as described in the ADNI procedures manual (<http://www.adni-info.org>), were also measured using Elecsys CSF immunoassays on a cobas e 601 analyzer at the University of Pennsylvania.³² ADNI CSF data (versions 2021-01-04 and 2019-07-29) were downloaded in early 2022 from the ADNI database (<https://ida.loni.usc.edu>) with corresponding participant demographics such as age, sex, diagnosis, and APOE genotypes. Data were verified to be the most currently available as of September 7, 2022.

The Elecsys CSF immunoassays had measuring ranges of 200–1700 pg/mL for A β 42, 0.006–40.3 ng/mL for A β 40 (specific for lot used), and 8–120 pg/mL for ptau181.^{31,32} Performance of the assays above these technical limits had not been formally established. Therefore, we only analyzed values within the technical limits. CSF A β 42/40 and ptau181/A β 42 ratios were derived from the CSF A β 42, A β 40, and ptau181 values.

2.4 | Statistical analyses

There were 1045 individuals available for analyses in WADRC ($n = 380$), WRAP ($n = 238$), and ADNI ($n = 427$). Initial analyses used WADRC and WRAP combined data, then replication analyses were performed using ADNI data. Statistical analyses were performed in R (version 4.2.0).³³ Sample characteristics were compared between studies using analysis of variance for continuous measures and chi-square for categorical measures.

Associations between CSF biomarker (A β 42/40 ratio, ptau181, or ptau181/A β 42 ratio) and APOE variable (APOE-npscore, APOE4-

status, or $\epsilon 4$ -count) were each tested using linear mixed-effects regression in the lmerTest R package (version 3.1.1-3)³⁴ with random intercepts for each participant to account for multiple LP visits. Self-reported sex, clinical diagnosis (CU, MCI-AD, dementia-AD), and linear and quadratic terms for mean centered age at LP were entered as fixed covariates. CSF ptau181 values and the ptau181/A β 42 ratio were \ln -transformed and standardized within study. Residual diagnostics, used to verify covariate selection and check model assumptions, were performed using the DHARMa R package (version 0.4.5).³⁵ To determine goodness-of-fit and quantify differences in model fit between APOE-npscore, APOE4-status (0, 1), and $\epsilon 4$ -count (0, 1, 2), we compared the Akaike information criterion (AIC), Bayesian information criterion (BIC), and the proportion of variance explained by models differing only by the APOE variable as predictor. Pseudo- R^2 statistics were calculated using the MuMIn R package (version 1.47.1)³⁶ and the marginal R^2 were compared to determine the difference in variance attributable to the fixed effects portion of each model. Relative improvement between models was calculated using the ratio of marginal R^2 values.

Other R packages used included kableExtra (version 1.3.4),³⁷ tableone (version 0.13.2),³⁸ and sjPlot (version 2.8.11).³⁹

3 | RESULTS

3.1 | Participant characteristics by study

The characteristics of participants in WADRC, WRAP, and ADNI are shown by study in Table 1. Comparisons between WADRC and WRAP are provided in Table S1 and characteristics by ADNI protocol (ADNI1, ADNI2, ADNIGO, and ADNI3) are shown in Table S2. Participant characteristics are based on the most recent LP visit; information about multiple LP visits and biomarker values are provided based on longitudinal data (1566 CSF samples). Table S3 shows characteristics by study (WADRC, WRAP, and ADNI) for a subset of CU participant samples used in sensitivity analyses.

All studies included predominantly non-Hispanic white (NHW) and mostly female individuals. There were significant differences in clinical diagnosis across studies. WRAP was comprised of CU individuals with a few MCI-AD, both WADRC and ADNI had similar numbers of dementia-AD, and ADNI had the largest proportion of MCI-AD. Mean APOE-npscores were lower in WRAP (0.54 ± 1.02) than WADRC (0.79 ± 1.20) and ADNI (0.75 ± 1.18). Less than 14% of WADRC, almost 27% of WRAP, and less than 17% of ADNI participants had 2 or more LPs with a mean difference ~ 2.5 years between visits. Mean age at LP across longitudinal samples was similar in WADRC and WRAP (~ 63 years) but older in ADNI (73 years). ADNI participants had significantly higher levels of both CSF A β 42 (913 ± 372 pg/mL) and ptau181 (24.6 ± 12.9 pg/mL) than WADRC (A β 42: 826 ± 364 pg/mL; ptau181: 20.3 ± 11.4 pg/mL) and WRAP (A β 42: 861 ± 354 pg/mL; ptau181: 18.6 ± 6.62 pg/mL). Scatterplots of age at LP against CSF A β 42, A β 42/40 ratio, and ptau181 values by study are shown in Figure 1.

TABLE 1 Cohort demographics at most recent lumbar puncture and longitudinal biomarkers.

	Overall	WADRC	WRAP	ADNI
<i>n</i> (individual participants)	1045	380	238	427
Summary diagnosis, <i>n</i> (%)***				
Dementia-AD	95 (9.1)	48 (12.6)	0 (0.0)	47 (11.0)
MCI-AD	168 (16.1)	42 (11.1)	3 (1.3)	123 (28.8)
CU	782 (74.8)	290 (76.3)	235 (98.7)	257 (60.2)
Female, <i>n</i> (%)*	622 (59.5)	231 (60.8)	156 (65.5)	235 (55.0)
Self-reported race, <i>n</i> (%)				
NHB	34 (3.3)	14 (3.7)	4 (1.7)	16 (3.7)
Other	20 (1.9)	3 (0.8)	5 (2.1)	12 (2.8)
NHW	991 (94.8)	363 (95.5)	229 (96.2)	399 (93.4)
APOE genotype, <i>n</i> (%)*				
ε2ε2	1 (0.1)	1 (0.3)	0 (0.0)	0 (0.0)
ε2ε3	91 (8.7)	35 (9.2)	24 (10.1)	32 (7.5)
ε3ε3	535 (51.2)	177 (46.6)	131 (55.0)	227 (53.2)
ε2ε4	27 (2.6)	15 (3.9)	5 (2.1)	7 (1.6)
ε3ε4	308 (29.5)	116 (30.5)	71 (29.8)	121 (28.3)
ε4ε4	83 (7.9)	36 (9.5)	7 (2.9)	40 (9.4)
APOE-npscore, mean (SD)*	0.72 (1.16)	0.79 (1.20)	0.54 (1.02)	0.75 (1.18)
Longitudinal biomarkers				
<i>n</i> samples	1566	498	515	553
LP visit, <i>n</i> (%)***				
1	1045 (67.0)	380 (76.3)	238 (46.2)	427 (77.2)
2	295 (18.8)	65 (13.1)	137 (26.6)	93 (16.8)
3	145 (9.3)	26 (5.2)	93 (18.1)	26 (4.7)
4	66 (4.2)	18 (3.6)	41 (8.0)	7 (1.3)
5	12 (0.8)	6 (1.2)	6 (1.2)	0 (0.0)
6	2 (0.1)	2 (0.4)	0 (0.0)	0 (0.0)
7	1 (0.1)	1 (0.2)	0 (0.0)	0 (0.0)
Age at LP (years), mean (SD)***	66.94 (9.15)	63.26 (9.31)	63.97 (6.93)	73.01 (7.55)
Years between visits, mean (SD)***	2.55 (1.51)	2.57 (2.22)	2.42 (1.12)	2.84 (1.41)
Aβ42 (pg/mL), mean (SD)**	865 (364)	826 (364)	861 (354)	913 (372)
Aβ42/40 ratio, mean (SD)***	0.06 (0.02)	0.06 (0.02)	0.06 (0.02)	0.06 (0.02)
ptau181 (pg/mL), mean (SD)***	21.3 (11.1)	20.3 (11.4)	18.6 (6.62)	24.6 (12.9)
ptau181/Aβ42 ratio, mean (SD)***	0.03 (0.03)	0.03 (0.03)	0.03 (0.02)	0.04 (0.03)

Note: *p*-values from Pearson's Chi-squared test; Wilcoxon rank sum test.

Abbreviations: ADNI, Alzheimer's Disease Neuroimaging Initiative; APOE, apolipoprotein E; APOE-npscore, neuropathology-based APOE genetic risk score; Aβ42, CSF amyloid-beta1-42; Aβ42/40, CSF amyloid-beta1-42 /CSF amyloid-beta1-40 ratio; CU, cognitively unimpaired; Dementia-AD, dementia suspected due to AD; LP, lumbar puncture; MCI-AD, mild cognitive impairment suspected due to AD; NHB, self-reported non-Hispanic Black; NHW, self-reported non-Hispanic white; ptau/Aβ42 ratio, CSF phosphorylated tau 181/CSF amyloid-beta1-42 ratio; ptau181, CSF phosphorylated tau 181; SD, standard deviation; WADRC, Wisconsin Alzheimer's Disease Research Center; WRAP, Wisconsin Registry for Alzheimer's Prevention.

p* < 0.05; *p* < 0.01; ****p* < 0.001.

3.2 | Using ε4-count provided a better model fit than APOE4-status and explained more variance in CSF AD endophenotypes

Some researchers use ε4-count instead of the binary APOE4-status, so we tested if ε4-count provides a better model fit than APOE4-status.

In our preliminary analyses of WADRC and WRAP, APOE4-status and ε4-count were associated with CSF Aβ42/40 ratio ($\beta = -0.012$, $p = 3.98 \times 10^{-21}$ and $\beta = -0.010$, $p = 1.04 \times 10^{-23}$, respectively), ptau181 ($\beta = 0.258$, $p = 6.49 \times 10^{-4}$ and $\beta = 0.238$, $p = 9.20 \times 10^{-5}$, respectively), and ptau181/Aβ42 ratio ($\beta = 0.524$, $p = 2.52 \times 10^{-14}$ and $\beta = 0.460$, $p = 7.42 \times 10^{-17}$, respectively); however, models using

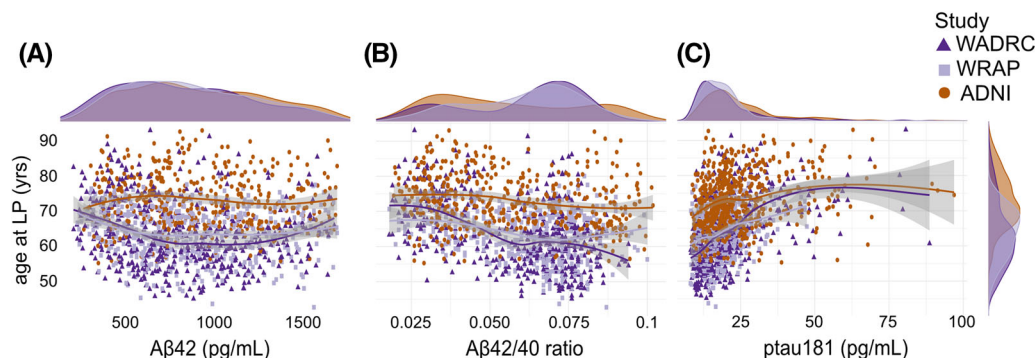


FIGURE 1 Cerebrospinal fluid (CSF) biomarker levels by age at time of lumbar puncture (LP). Scatterplots of the age at time of LP against CSF A β 42 (A), A β 42/40 ratio (B), and ptau181 (C) with density plots for each biomarker on top and a density plot for age at LP for all three panels on the right-hand side of (C). Each point represents an individual CSF sample. Densities, loess curves, and points are all color-coded by study as shown in the legend; triangle-shaped points indicate CSF sample data from Wisconsin Alzheimer's Disease Research Center (WADRC), square-shaped points indicate CSF sample data from Wisconsin Registry for Alzheimer's Prevention (WRAP), and circle-shaped points indicate CSF sample data from Alzheimer's Disease Neuroimaging Initiative (ADNI).

TABLE 2 Linear-mixed effects model comparisons between APOE genetic variables

	CSF A β 42/40 ratio				CSF ptau181				CSF ptau181/A β 42 ratio			
	n	R ²	AIC	BIC	n	R ²	AIC	BIC	n	R ²	AIC	BIC
WADRC/WRAP												
APOE4-status	948	0.344	-5728	-5684	971	0.203	1641	1685	924	0.358	1781	1825
ϵ 4-count	948	0.355	-5740	-5696	971	0.208	1637	1681	924	0.370	1770	1813
APOE-npscore	948	0.360	-5746	-5702	971	0.210	1635	1679	924	0.378	1761	1805
ADNI												
APOE4-status	423	0.315	-2285	-2248	548	0.200	1156	1195	418	0.344	909	945
ϵ 4-count	423	0.359	-2309	-2272	548	0.208	1152	1190	418	0.394	880	917
APOE-npscore	423	0.375	-2318	-2282	548	0.218	1147	1186	418	0.409	872	908

Abbreviations: ADNI, Alzheimer's Disease Neuroimaging Initiative; AIC, Akaike Information Criterion; APOE4-status, APOE4-carrier status (0, 1); APOE-npscore, APOE neuropathology-based score; A β 42/40, CSF amyloid-beta1-42/amyloid-beta1-40 ratio; BIC, Bayesian Information Criterion; CSF, cerebrospinal fluid; n, number of observations; ptau/A β 42 ratio, CSF phosphorylated tau 181/CSF amyloid-beta1-42 ratio; ptau181, CSF phosphorylated tau 181; R², marginal R² (variation explained by fixed effects); WADRC, Wisconsin Alzheimer's Disease Research Center; WRAP, Wisconsin Registry for Alzheimer's Prevention; ϵ 4-count, number of APOE ϵ 4 alleles (0, 1, 2).

ϵ 4-count explained more variance than APOE4-status with 3.2% relative increase in CSF A β 42/40 ratio (marginal R² = 0.355 vs. 0.344), 2.5% relative increase in ptau181 (marginal R² = 0.208 vs. 0.203), and 3.4% increase in ptau181/A β 42 ratio (marginal R² = 0.370 vs. 0.358). Model fit comparisons are shown in Table 2 and detailed regression results in Tables S4–S6.

As shown in Table 2, our findings were replicated in ADNI. Models with ϵ 4-count explained more variance than APOE4-status with 14% relative increase explained in CSF A β 42/40 ratio (marginal R² = 0.359 vs. 0.315), 4% increase in ptau181 (marginal R² = 0.208 vs. 0.200), and 14.5% increase in ptau181/A β 42 ratio (marginal R² = 0.394 vs. 0.344). Detailed results are shown in Tables S7–S9.

3.3 | APOE-npscore provided a better model fit than ϵ 4-count and explained more variance in CSF AD endophenotypes

After determining that ϵ 4-count provided a better model fit than APOE4-status, we tested if the APOE-npscore improved the model fit even more than ϵ 4-count. In the WADRC and WRAP, APOE-npscore was associated with CSF A β 42/40 ratio (β = -0.006, p = 6.63 \times 10⁻²⁵), ptau181 (β = 0.140, p = 2.35 \times 10⁻⁵), and ptau181/A β 42 ratio (β = 0.264, p = 1.09 \times 10⁻¹⁸). Models using APOE-npscore explained more variance than ϵ 4-count with a relative increase of 1.4% variance explained in CSF A β 42/40 ratio (marginal R² = 0.360 vs. 0.355), 1% increase in ptau181 (marginal R² = 0.210 vs. 0.208), and 2.2% increase

explained in ptau181/A β 42 ratio (marginal $R^2 = 0.378$ vs. 0.370). As shown in Table 2, both AIC and BIC model selection criteria consistently supported models using the APOE-npscore over models using the ϵ 4-count and models using the APOE4-status. Detailed results are shown in Tables S4–S6.

Our findings were replicated in the ADNI. APOE-npscore explained more variance than ϵ 4-count with a relative increase of 4.5% explained in CSF A β 42/40 ratio (marginal $R^2 = 0.375$ vs. 0.359), 4.8% increase in ptau181 (marginal $R^2 = 0.218$ vs. 0.208), and 3.8% increase in ptau181/A β 42 ratio (marginal $R^2 = 0.409$ vs. 0.394). Detailed results are shown in Tables S7–S9.

3.4 | APOE-npscore provided a better model fit in a subset of cognitively unimpaired participants

Previous studies have reported preclinical effects of APOE4-status and ϵ 4-count on CSF AD biomarkers.^{40,41} To test if the APOE-npscore could provide a better model fit than APOE4-status and ϵ 4-count when analyzing CSF AD endophenotypes before cognitive symptoms appear, we reran the analyses in a subset of longitudinal samples from participants who were CU at LP (described in Table S3). There were significant associations between CSF A β 42/40 ratio and the APOE-npscore ($\beta = -0.005$, $p = 1.06 \times 10^{-18}$), APOE4-status ($\beta = -0.011$, $p = 1.47 \times 10^{-16}$), and ϵ 4-count ($\beta = -0.010$, $p = 4.63 \times 10^{-18}$); between ptau181 and APOE-npscore ($\beta = 0.110$, $p = 1.52 \times 10^{-3}$), APOE4-status ($\beta = 0.217$, $p = 4.68 \times 10^{-3}$), and ϵ 4-count ($\beta = 0.191$, $p = 3.32 \times 10^{-3}$); and between ptau181/A β 42 ratio and APOE-npscore ($\beta = 0.241$, $p = 1.17 \times 10^{-13}$), APOE4-status ($\beta = 0.491$, $p = 1.04 \times 10^{-11}$), and ϵ 4-count ($\beta = 0.437$, $p = 7.85 \times 10^{-13}$). There was also more variance explained by the APOE-npscore than the APOE4-status with a relative increase of 3.7% in CSF A β 42/40 ratio (marginal $R^2 = 0.222$ vs. 0.214), 1.4% in CSF ptau181 (marginal $R^2 = 0.150$ vs. 0.148), and 4.9% in CSF ptau181/A β 42 ratio (marginal $R^2 = 0.216$ vs. 0.206). APOE-npscore also explained more variance than ϵ 4-count with a relative increase of 1% in CSF A β 42/40 ratio (marginal $R^2 = 0.222$ vs. 0.220) and 1.4% in ptau181/A β 42 ratio (marginal $R^2 = 0.216$ vs. 0.213), but no difference in ptau181 (marginal $R^2 = 0.150$ vs. 0.150). Both AIC and BIC model selection criteria consistently supported models using the APOE-npscore (A β 42/40 ratio: AIC = -5175, BIC = -5137; ptau181: AIC = 1304, BIC = 1342; ptau181/A β 42 ratio: AIC = 1512, BIC = 1550) over models using the ϵ 4-count (A β 42/40 ratio: AIC = -5172, BIC = -5134; ptau181: AIC = 1305, BIC = 1343; ptau181/A β 42 ratio: AIC = 1516, BIC = 1553) and models using the APOE4-status (A β 42/40 ratio: AIC = -5165, BIC = -5127; ptau181: AIC = 1306, BIC = 1344; ptau181/A β 42 ratio: AIC = 1521, BIC = 1558). Detailed results are shown in Tables S10–S12.

These findings were replicated in a subset of samples from participants who were CU in ADNI (described in Table S3). CSF A β 42/40 ratio was associated with the APOE-npscore ($\beta = -0.009$, $p = 8.13 \times 10^{-12}$), APOE4-status ($\beta = -0.018$, $p = 5.94 \times 10^{-9}$), and ϵ 4-count ($\beta = -0.016$, $p = 6.78 \times 10^{-11}$). CSF ptau181 was associated with APOE-npscore

($\beta = 0.181$, $p = 3.74 \times 10^{-4}$), APOE4-status ($\beta = 0.324$, $p = 4.04 \times 10^{-3}$), and ϵ 4-count ($\beta = 0.298$, $p = 1.54 \times 10^{-3}$). The CSF ptau181/A β 42 ratio was associated with APOE-npscore ($\beta = 0.335$, $p = 8.55 \times 10^{-11}$), APOE4-status ($\beta = 0.602$, $p = 3.26 \times 10^{-7}$), and ϵ 4-count ($\beta = 0.583$, $p = 8.93 \times 10^{-10}$). There was more variance explained by the APOE-npscore than the APOE4-status with a relative increase of 20.5% in CSF A β 42/40 ratio (marginal $R^2 = 0.264$ vs. 0.219), 9% in CSF ptau181 (marginal $R^2 = 0.182$ vs. 0.167), and 22.5% in CSF ptau181/A β 42 ratio (marginal $R^2 = 0.299$ vs. 0.244). APOE-npscore also explained more variance than ϵ 4-count with a relative increase of 6.5% in CSF A β 42/40 ratio (marginal $R^2 = 0.264$ vs. 0.248), 5.8% in ptau181 (marginal $R^2 = 0.182$ vs. 0.172), and 6% in ptau181/A β 42 ratio (marginal $R^2 = 0.299$ vs. 0.282). Both AIC and BIC model selection criteria consistently supported models using the APOE-npscore (A β 42/40 ratio: AIC = -1422, BIC = -1387; ptau181: AIC = 657, BIC = 696; ptau181/A β 42 ratio: AIC = 508, BIC = 543) over models using the ϵ 4-count (A β 42/40 ratio: AIC = -1418, BIC = -1382; ptau181: AIC = 660, BIC = 699; ptau181/A β 42 ratio: AIC = 513, BIC = 548) and models using the APOE4-status (A β 42/40 ratio: AIC = -1409, BIC = -1373; ptau181: AIC = 662, BIC = 701; ptau181/A β 42 ratio: AIC = 525, BIC = 560). Detailed results are shown in Tables S13–S15.

4 | DISCUSSION

We report here a method for translating APOE haplotype (ϵ 2 ϵ 2, ϵ 2 ϵ 3, ϵ 2 ϵ 4, ϵ 3 ϵ 3, ϵ 3 ϵ 4, and ϵ 4 ϵ 4) into a pseudo-continuous measure reflecting APOE genetic risk for autopsy-confirmed AD. We demonstrated the APOE-npscore provides a better model fit than dichotomous APOE4-status, and even ϵ 4-count, in statistical models of CSF endophenotypes for AD neuropathology (A β 42/40 ratio, ptau181, and ptau181/A β 42 ratio). Although some of the statistical improvements we reported in this study were small, there are several benefits of using APOE-npscore in AD-related research. Not only does it more accurately represent corresponding risk for each haplotype, the APOE-npscore more closely reflects the genetic effect of APOE on AD neuropathology. It allows researchers to account for effects of ϵ 2 and ϵ 4 in one variable. As an improvement to APOE clinical risk scores, by using the results of a large autopsy-confirmed AD case-control study,¹² we minimize bias from misclassified dementia cases and preclinical controls.

CSF biomarkers change years before cognitive symptoms appear and are not only correlated with AD neuropathology but also with APOE.^{1,22,42} As expected, all the methods we tested for encoding APOE genotype were significantly associated with CSF A β 42/40 ratio, ptau181, and ptau181/A β 42 ratio. In all three AD endophenotypes, APOE-npscore appeared to provide a better model fit than APOE4-status and ϵ 4-count and there was more variance explained by the APOE-npscore models, consistently observed in WADRC, WRAP, and ADNI as well as subsets of CU individuals. These findings suggest that studies of preclinical AD, such as personalized prevention trials, could benefit from using the APOE-npscore instead of separating groups by APOE4-status. The APOE-npscore provides a better model

fit for CSF AD endophenotypes and likely would do the same for trial endpoints. The APOE-npscore could easily be translated to APOE4-status or ϵ 4-count if needed, but the opposite isn't necessarily true. Using the APOE-npscore in trial design would require genotyping both APOE SNPs (rs429358 for ϵ 4, rs7412 for ϵ 2) and place greater emphasis on recruiting rarer ϵ 2 carriers than designs that use APOE4-status, but the added benefit is that the precision of the APOE-npscore could help personalize treatment.

There are important limitations in this study to consider for future research. The Reiman et al. study that provided OR used to derive the APOE-npscore consisted of NHW individuals¹² and the cohorts included in our analyses comprised > 94% NHW participants. With reported disparities in biomarker outcomes and in APOE genetic risk, there is evidence our findings may not translate directly to other populations.⁴³⁻⁵⁴ Self-identified non-Hispanic Black (NHB) individuals are at a greater risk of AD dementia than NHW; however, even though APOE ϵ 4 is more common in African genetic ancestry (rs429358 MAF: 0.26 in AFR vs. 0.14 in EUR), studies suggest APOE ϵ 4 has a weaker effect, or no effect, on AD dementia in NHB individuals.⁴⁵⁻⁴⁹ Linkage disequilibrium structure of the APOE gene region varies across genetic ancestries,⁵⁰ and studies show there are genetic haplotypes predominantly present in African genetic ancestry that may explain some of the differences in APOE genetic effect on AD risk.^{3,51} Disparities in the APOE ϵ 4 genetic effect on AD risk have been observed in other populations. Although studies of Chinese patients show that APOE ϵ 4 increases risk for AD similar to NHW,^{52,53} a study of neuroimaging and cognitive testing from 811 American Indians in the Strong Heart Study found no evidence of increased risk from APOE ϵ 4.⁵⁴ Racial disparities in these examples and other AD biomarker studies demonstrate that although race is not a biological construct, the biological outcomes of interest are confounded by racial disparities in study recruitment and research.^{55,56} Further research with diverse cohorts will be necessary to test and adapt the APOE-npscore to be useful for a much broader group of people, many of whom are at an even greater risk for AD dementia than NHW individuals.

Another limitation is that we were unable to evaluate APOE-npscore performance directly because the training data from Reiman et al. was available as summary statistics, not individual-level data, and our current testing data do not have the same phenotype (autopsy-confirmed AD diagnosis) for validating the APOE-npscores. Future research to evaluate the APOE-npscore will require an independent dataset with individual-level autopsy data; ideally a diverse cohort that could help fine-tune the APOE-npscore to be useful for the broader population. Our findings in WRAP and WADRC were replicated in the independent ADNI cohort, which is promising. Since the APOE-npscore is easily derived from the APOE ϵ 2/ ϵ 3/ ϵ 4 haplotypes, we anticipate several studies will be able to benefit from the improved method for accounting for APOE genetic risk.

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CONFLICT OF INTEREST STATEMENT

I.S. is a full-time employee and shareholder of Roche Diagnostics International Ltd., Rotkreuz, Switzerland. G.K. and N.W. are full-time employees of Roche Diagnostics GmbH, Penzberg, Germany. S.C.J. serves as a consultant to Roche Diagnostics and Prothena and receives research support from Cerveau Technologies for unrelated work. H.Z. has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Passage Bio, Pinteon Therapeutics, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). K.B. has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, BioArctic, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Ono Pharma, Pharmatrophix, Prothena, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, outside the work presented in this paper. Other authors have no competing interests to declare. Author disclosures are available in the [supporting information](#).

DATA AVAILABILITY STATEMENT

All ADNI data are available through the LONI Imaging & Data Archive. Interested scientists may apply for access on the ADNI web-

site (<http://adni.loni.usc.edu/data-samples/access-data/>). Interested scientists may apply to access data from the WADRC and WRAP through the website (<https://www.adrc.wisc.edu/apply-resources>).

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SUPPORTING INFORMATION

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

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