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RESEARCH ARTICLE

# Epigenome-wide association study of cerebrospinal fluid–based biomarkers of Alzheimer's disease in cognitively normal individuals

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## Abstract

**INTRODUCTION:** Cerebrospinal fluid (CSF) biomarkers of Alzheimer's disease (AD) are reliable predictors of future AD risk. We investigated whether pre-clinical changes in AD CSF biomarkers are reflected in blood DNA methylation (DNAm) levels in cognitively normal participants.

**METHODS:** We profiled blood-based DNAm with the EPIC array in participants without a diagnosis of cognitive impairment in the Emory Healthy Brain Study (EHBS; $N = 495$), Alzheimer's Disease Neuroimaging Initiative ($N = 122$), and Parkinson's Progression Markers Initiative ($N = 118$) cohorts. Their CSF amyloid beta 42, total tau (t-tau), and phosphorylated tau181 levels were quantified using Elecsys immunoassays. We conducted epigenome-wide association studies to assess associations between DNAm and CSF biomarkers of AD.

**RESULTS:** In EHBS, no loci were Bonferroni significant after adjusting for confounding factors. In the meta-analysis of all three cohorts, DNAm in cg22976567 (*LMNA*) was significantly associated with higher CSF t-tau levels.

Aliza P. Wingo and Thomas S. Wingo contributed equally to this study.

**DISCUSSION:** Our study showed little evidence of an association between differential blood-based DNAm and pre-clinical AD CSF biomarkers.

KEYWORDS
Alzheimer's disease, amyloid, biomarker, blood, cerebrospinal fluid, DNA methylation, epigenetics, epigenome-wide association study

**Highlights**

- We conducted one of the largest ($n = 735$) blood DNA methylation (DNAm) studies of Alzheimer's disease cerebrospinal fluid (AD CSF) biomarkers.
- This is the first epigenome-wide association study in cognitively normal participants examining AD CSF biomarkers.
- Limited associations between blood DNAm and AD CSF biomarkers were identified.

## 1 | BACKGROUND

Alzheimer's disease (AD) and related dementias (ADRD) are the sixth leading cause of death affecting > 6 million people in the United States, and the number is projected to grow to 13.8 million by 2060.[1,2] Individuals living with AD/ADRD experience progressively worsening cognitive impairment that compromises their quality of life and often requires long-term day-to-day support and care.[2]

AD has a long asymptomatic stage that can be detected in brain imaging and cerebrospinal fluid (CSF).[3] In 2018, the National Institute on Aging and Alzheimer's Association drew a distinction between the underlying pathologic process of AD and the clinical signs and symptoms those pathologic changes may cause (i.e., subjective cognitive impairment, mild cognitive impairment [MCI], and dementia). The rationale for this distinction is the emergence of reliable AD biomarkers that can measure amyloid beta (A$\beta$) deposition, pathologic tau, and neurodegeneration (collectively termed the ATN framework), which are detectable up to 30 years *before* symptomatic cognitive impairment.[4] Identifying patients who are on the path of developing dementia is essential for early intervention and treatment. Early biomarkers of AD, for example, CSF biomarkers,[5–9] provide an opportunity to detect the first signs of disease.

In recent years, there has been a substantial increase in studies characterizing epigenetic mechanisms in AD, mainly focused on DNA methylation (DNAm).[10] In the brain, epigenetic studies suggest robust associations between DNAm and AD,[11] including differential DNAm in the amyloid precursor protein (*APP*),[12] microtubule-associated protein tau (*MAPT*),[12] apolipoprotein E (*APOE*) promoter region,[13] homeobox A3 (*HOXA3*),[14] interleukin-1 beta (*IL-1β*),[15] interleukin-6 (*IL-6*),[15] and claudin-5 (*CLDN5*) genes.[16] While identifying AD-related epigenetic signatures in the brain can help unravel some biological mechanisms involved in the pathophysiology of AD, understanding epigenetic changes in the blood is crucial for the development of new molecular indicators for AD, given that blood collection is a minimally invasive routine procedure, which can be performed while patients are alive.[17]

Several blood-based DNAm markers have been identified in association with AD, including differential DNAm in the homeobox B6 (*HOXB6*) gene, [18] the oxytocin (*OXT*) gene,[19] and the adenosine deaminase RNA–specific B2 (*ADARB2*) gene.[20] One study of 202 participants from the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort identified a number of associations between blood DNAm and CSF biomarkers comparing 123 cognitively normal participants to 79 AD participants.[21] Another study identified 12 DNAm sites in blood associated with CSF biomarkers among 885 participants included in the European Medical Information Framework for AD (EMIF-AD) study[22]—most of them diagnosed with MCI or AD. However, it is unknown whether differential DNAm is also associated with pre-clinical stages of AD.

To address this knowledge gap, we investigated whether blood DNAm was associated with CSF A$\beta$42, total tau (t-tau), and phosphorylated tau (p-tau) biomarkers in 735 cognitively normal participants enrolled in the Emory Healthy Brain Study (EHBS), the ADNI cohort, and the Parkinson's Progression Markers Initiative (PPMI) cohort. We hypothesized that pre-clinical changes in CSF biomarkers of AD will be mirrored in the blood epigenome, which could help us to better understand the biological mechanisms underlying pre-clinical changes of AD. To test this hypothesis, we conducted independent epigenome-wide association studies (EWAS) in each cohort followed by an epigenome-wide meta-analysis to assess the association between blood-based DNAm and CSF biomarkers of AD.

## 2 | METHODS

### 2.1 | Study population

Our study is based on data from 495 individuals from the EHBS ($N = 450$ White participants, $N = 45$ Black participants), 122 White individuals from ADNI, and 118 White individuals from PPMI classified as controls without a diagnosis of cognitive impairment (i.e., MCI,

AD, or other dementias) at blood draw. Only one ADNI participant self-identified as Black and was excluded from the analysis.

The EHBS is a prospective research study focusing on preclinical biomarkers and the cognitive health of older adults. The EHBS is nested within the Emory Healthy Aging Study (EHAS) and includes participants from the metro-Atlanta region in Georgia, USA. The EHBS was launched in 2016 and the primary aim is to characterize biological, physiological, and psychosocial factors associated with normal and abnormal aging through assessment of the central nervous system among adults 45 to 75 years old who were free of cognitive impairment in addition to several other chronic conditions (e.g., congestive heart failure, multiple sclerosis, human immunodeficiency virus) at enrollment; more details on recruitment and eligibility have been published elsewhere.[23] All participants completed an online consent process prior to enrollment and provided informed consent. The study was approved by the Emory University Institutional Review Board (IRB).

ADNI is a longitudinal, observational study designed to collect and validate biomarkers for AD. ADNI was launched in 2003 as a public–private partnership with a primary goal to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and AD for clinical trials. Participants must be between the ages of 55 and 90 and be in good general health, with or without memory problems or concerns. Participant recruitment for ADNI is approved by the IRB of each participating site. All ADNI participants undergo standardized diagnostic assessment that renders a clinical diagnosis of either control, MCI, or AD using standard research criteria.[24]

PPMI is an observational, international, multicenter study designed to establish biomarker-defined cohorts, identify Parkinson's disease (PD) progression biomarkers to improve understanding of disease etiology and course, and to provide the necessary tools to enhance the likelihood of success of PD disease-modifying therapeutic trials.[25] PPMI was launched in 2010, recruiting people with and without PD (healthy controls). In this study, we included the healthy controls from the PPMI. Healthy controls are ≥ 30 years; have no clinically significant neurological, medical, or psychiatric conditions; no first-degree relative with PD; and normal dopamine transporter single-photon emission computed tomography imaging by visual inspection. The study was conducted in accordance with the Declaration of Helsinki and the Good Clinical Practice guidelines after approval of the local ethics committees of the participating centers.[25]

## 2.2 | CSF AD biomarker measurements

In the EHBS, ADNI, and PPMI cohorts, CSF biospecimens were collected via lumbar puncture using standardized research protocols.[26] In all three cohorts, concentrations of Aβ42, t-tau, and p-tau181 (herein referred to as p-tau) in CSF were quantified using the first-generation Elecsys ElectroChemiLuminescense Immunoassay (ECLIA) platform on a cobas e 601 analyzer (F. Hoffman-La Roche Ltd) in different laboratories, as previously described.[27–29]

### RESEARCH IN CONTEXT

1. **Systematic review**: Alzheimer's disease (AD) has a long asymptomatic stage, commonly referred to as the preclinical period. Cerebrospinal fluid (CSF) biomarkers of AD can measure amyloid beta deposition, pathologic tau, and neurofilament light chain (NfL), which are detectable up to 30 years before cognitive impairment. Several blood-based DNA methylation (DNAm) markers have been identified in association with AD, but it is unknown whether differential DNAm is also associated with preclinical stages of AD.
2. **Interpretation**: Our work presents one of the largest epigenome-wide association studies of AD CSF biomarkers and the first that was conducted among cognitively normal individuals (735 individuals from three independent cohorts). Overall, there was only weak evidence of an association between differential blood DNAm and AD CSF biomarkers in the pre-clinical stages of AD.
3. **Future directions**: Future studies should include additional biomarkers such as CSF biomarkers of neuroinflammation (chitinase-3-like protein 1 [YKL-40]), NfL, or blood-based AD biomarkers, which might show stronger associations with blood-based DNAm.

## 2.3 | DNAm data quality control

In EHBS, ADNI, and PPMI, DNAm was measured using the Illumina Infinium HumanMethylationEPIC BeadChip version 1, which quantified > 850,000 CpGs sites. DNAm preprocessing and sample quality control followed prior work[30,31] as described previously[32] and was done separately for EHBS, ADNI, and PPMI.

In EHBS and ADNI, sample quality control measures included assessment of: (1) 17 technical parameters with R package ewastools,[33] including array staining, extension, hybridization, target removal, specificity, bisulfite conversion; (2) estimates of the methylated and unmethylated intensities with the getQC function from minfi[34] using default parameters; (3) agreement between predicted (inferred from DNAm intensities of the sex chromosome using the minfi R package) and recorded sex; (4) poor probe detection determined by if a samples had > 1% probes with detection $p$ value > 0.01; (5) low beadcount if 1% probes had beadcount < 3; (6) outlier detection by function outlyx from R package wateRmelon[35] using default parameter. After sample quality checks, probe level quality control was performed by removing XY probes and removing probes with bad detection $p$ value ($p$ value > 0.01) or with bead count < 3. Probes identified as poorly performing in > 1% samples were removed from all samples. Probes that were cross-hybridizing/cross-reactive and occurred over polymorphic sites, defined by Pidsley annotation,[36] were removed. Subsequently, probe-level normalization was done

in two steps. First, we normalized for color bias, background noise, and dye-bias as implemented with preprocessNoob function from the minfi R package 1.42.0(37). Second, we applied the $\beta$-mixture quantile normalization (BMIQ) procedure in the wateRmelon R package 2.2.0[35] to normalize beta value distributions of type 1 and type 2 design probes in the Illumina arrays. To account for batch effects, including chip ID, chip position, and plate effects, we used the function ComBat() from the sva R package 3.48.0[35] using default parameters.

In PPMI, quality control was performed using the minfi R package.[37] First, detection *p* value was computed using detectionP; samples with a mean detection *p* value > 0.01 were excluded (two samples were excluded). The sex of each sample was then predicted using getSex function, and samples discordant between clinically reported sex and methylation predicted sex were excluded (two samples were excluded). After sex discrepancy and detection *p* value sample exclusions, multidimensional scaling was performed using mdsPlot; one sample outlier was excluded. Next, detected CpG (0.01) within Genome Studio was used to determine specific sentrix arrays to be excluded for subjects that were processed more than once.[38] Probes with a detection *p* value > 0.01 in ≥ 20% of samples were identified. Functional normalization was performed on the data using preprocessFunnorm followed by removal of 2769 identified, failed probes.[38]

After quality control, 661,869 probes and 495 unique samples remained for the analysis in the EHBS, 699,218 probes and 122 unique samples in ADNI, and 864,067 probes and 118 unique samples in PPMI.

## 2.4 | Epigenome-wide association study

Because the White EHBS participants ($N = 450$) were by far the largest sample and CSF AD biomarkers differ by race,[39] the White EHBS samples were used in our main analysis, followed by a replication of the top 10 CpGs in ADNI ($N = 122$), PPMI ($N = 118$), and in the Black EHBS participants ($N = 45$). We conducted independent EWAS of CSF AD biomarkers in EHBS (stratified by race), ADNI (all White), and PPMI (all White). An epigenome-wide meta-analysis of the White participants from EHBS, ADNI, and PPMI was conducted as a secondary analysis.

The main outcomes considered were t-tau, p-tau, and A$\beta$42/tau ratio, which were Box–Cox transformed using the car R package to improve normality. We also used ADNI-established thresholds[27] of CSF A$\beta$42 and p-tau to dichotomize individuals for each measure (i.e., A$\beta$42 ± and p-tau ±). The threshold for A$\beta$42 was 980 pg/ml, and threshold for p-tau181 was 21.8 pg/mL. We used ADNI-established thresholds of CSF A$\beta$42 < 980 pg/mL and p-tau181 > 21.8 pg/mL to categorize individuals as either positive or negative for the respective measure (A+T+, A−T+, A+T−, and A−T−). The thresholds were selected to maximize the concordance with positive A$\beta$ determined by positive florbetapir ($^{18}$F-AV-45) PET imaging.[28]

The association between DNAm beta values, which represent the ratio of the methylated probe intensity to the overall intensity (sum of methylated and unmethylated probe intensities) at a given CpG site, and CSF biomarkers (i.e., t-tau, p-tau, A$\beta$42/tau ratio, A$\beta$42 ± , p-tau ±) was assessed using robust linear regression models with CSF biomarkers as independent variable and DNAm beta values as the dependent variable, with models fit using rlm from the MASS R package. All models were adjusted for age at baseline, sex, smoking status (i.e., with or without smoking history; except for PPMI, because smoking history was only available for 16 participants), and estimated cell-type proportions. In EHBS and ADNI, cell type proportions were estimated using the FlowSorted.Blood.EPIC (version 1.8.0) R package 2.0.0,[40] resulting in estimated proportions for B lymphocytes, natural killer cells, CD4 + T lymphocytes, CD8 + T lymphocytes, monocytes, and neutrophils. In PPMI, cell type proportions were estimated using the Houseman algorithm,[41] resulting in estimated proportions for B lymphocytes, natural killer cells, CD4 + T lymphocytes, CD8 + T lymphocytes, monocytes, and granulocytes.

To meta-analyze individual CpG results from EHBS, ADNI, and PPMI, we used the inverse-variance weighted fixed-effects model, as implemented in METAL.[45] We used the Bonferroni threshold to account for multiple testing, resulting in a threshold of $p = 7.55 \times 10^{-8}$ (0.05/661,869) for the EWAS in EHBS and $p = 7.61 \times 10^{-8}$ (0.05/657,010) in the meta-analysis.

To evaluate the robustness of our findings, we conducted the following sensitivity analyses: First, we additionally adjusted our analyses for 0, 1, or 2 copies of the *APOE* $\varepsilon$4 allele to evaluate how genetic risk influences our results. Second, we conducted a random-effects meta-analysis to evaluate how any potential heterogeneity between the studies influences our results.

We also explored if we could find differentially methylated regions (DMRs) in relation to CSF AD biomarkers using the DMRff method.[43] We chose this method as it provides decent power for detecting DMRs and without an inflated Type I error rate.[44]

We conducted several secondary analyses for the top 10 CpG sites from the EWAS in EHBS and from the meta-analysis. First, we assessed the correlation between the DNAm beta values of the top CpGs across blood and brain tissue using the Blood–Brain Epigenetic Concordance (BECon) tool,[45] and the data from Braun et al. on the Gene Expression Omnibus Database (accession code GSE111165).[45] To further aid the interpretation of our top associations, we conducted a gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis using the R package missMethyl based on the top 1000 CpG sites with lowest raw *p* values for the EHBS EWAS and the meta-analysis results.[46]

# 3 | RESULTS

## 3.1 | Demographics

There were 495 EHBS participants, 122 White ADNI participants, and 118 PPMI participants (Table 1) who met our inclusion criteria of being cognitively normal with available blood DNAm and CSF AD biomarkers. On average, the EHBS and PPMI participants were approximately 10 years younger than the ADNI participants (mean age [standard deviation] EHBS White: 62.9 [6.89] years; EHBS Black: 61.7 [8.06] years; ADNI: 74.2 [5.97]; PPMI: 62.1 [10.5]). EHBS included

**TABLE 1** Characteristics of the participants from EHBS, ADNI, and PPMI.

| | EHBS (*N* = 495) | | ADNI (*N* = 122) | PPMI (*N* = 118) |
|---|---|---|---|---|
| **Race** | **White (*N* = 450)** | **Black (*N* = 45)** | **White (*N* = 122)** | **White (*N* = 118)** |
| **A. Characteristics** | | | | |
| Sex | | | | |
| Female | 316 (70.2%) | 37 (82.2%) | 62 (50.8%) | 33 (28.0%) |
| Male | 134 (29.8%) | 8 (17.8%) | 60 (49.2%) | 85 (72.0%) |
| Age | | | | |
| Mean (SD) | 62.9 (6.89) | 61.7 (8.06) | 74.2 (5.97) | 62.1 (10.5) |
| Median (min, max) | 63.6 (45.2, 77.0) | 59.5 (50.1, 77.7) | 73.5 (62.0, 89.6) | 63.2 (31.2, 82.7) |
| Smoking | | | | |
| No | 312 (69.3%) | 31 (68.9%) | 70 (57.4%) | 10 (8.5%) |
| Yes | 138 (30.7%) | 14 (31.1%) | 52 (42.6%) | 6 (5.1%) |
| Missing | 0 (0%) | 0 (0%) | 0 (0%) | 102 (86.4%) |
| *APOE* ε4 | | | | |
| 0 | 312 (69.3%) | 24 (53.3%) | 94 (77.0%) | 90 (76.3%) |
| 1 | 122 (27.1%) | 18 (40.0%) | 25 (20.5%) | 25 (21.2%) |
| 2 | 15 (3.3%) | 2 (4.4%) | 3 (2.5%) | 3 (2.5%) |
| Missing | 1 (0.2%) | 1 (2.2%) | 0 (0%) | 0 (0%) |
| **B. CSF AD biomarkers** | | | | |
| t-tau | | | | |
| Mean (SD) | 190 (68.0) | 155 (47.5) | 250 (88.1) | 202 (88.0) |
| Median (min, max) | 176 (80.0, 555) | 161 (80.2, 282) | 227 (123, 574) | 183 (82.0, 581) |
| p-tau | | | | |
| Mean (SD) | 16.9 (6.72) | 14.4 (4.67) | 22.9 (9.27) | 18.7 (9.48) |
| Median (min, max) | 15.4 (8.00, 61.5) | 14.9 (8.00, 24.5) | 20.0 (10.4, 60.0) | 16.7 (8.24, 73.6) |
| Missing | 0 (0%) | 0 (0%) | 0 (0%) | 6 (5.1%) |
| Aβ42/t-tau | | | | |
| Mean (SD) | 6.80 (2.04) | 6.71 (1.86) | 5.53 (2.34) | 5.26 (1.82) |
| Median (min, max) | 7.07 (0.69, 11.3) | 6.72 (2.17, 10.5) | 5.71 (0.99, 10.5) | 5.68 (0.71, 8.55) |
| Missing | 0 (0%) | 0 (0%) | 0 (0%) | 23 (19.5%) |
| Aβ42+/− | | | | |
| A− | 324 (72.0%) | 23 (51.1%) | 84 (68.9%) | 32 (27.1%) |
| A+ | 126 (28.0%) | 22 (48.9%) | 38 (31.1%) | 63 (53.4%) |
| Missing | 0 (0%) | 0 (0%) | 0 (0%) | 23 (19.5%) |
| p-tau+/− | | | | |
| T− | 365 (81.1%) | 41 (91.1%) | 69 (56.6%) | 82 (69.5%) |
| T+ | 85 (18.9%) | 4 (8.9%) | 53 (43.4%) | 30 (25.4%) |
| Missing | 0 (0%) | 0 (0%) | 0 (0%) | 6 (5.1%) |

Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; *APOE*, apolipoprotein E; CSF, cerebrospinal fluid; EHBS, Emory Healthy Brain Study; PPMI, Parkinson's Progression Markers Initiative; p-tau, phosphorylated tau; SD, standard deviation; t-tau, total tau.

more females than ADNI and PPMI, particularly among the Black participants (EHBS White: 70.2%; EHBS Black: 82.2%; ADNI: 50.8%; PPMI: 28.0%). Fewer EHBS participants had a history of tobacco smoking (EHBS White: 30.7%; EHBS Black: 31.1%) compared to ADNI participants (42.6%). Smoking information was missing for most PPMI participants (86.4% missing). EHBS participants had a higher prevalence of the *APOE* ε4 allele than ADNI and PPMI participants (Table 1).

In line with their older age, ADNI participants showed more signs of AD-related changes in CSF AD biomarkers compared to the EHBS and PPMI participants. Average levels of t-tau and p-tau were lower among EHBS and PPMI participants than among ADNI participants.

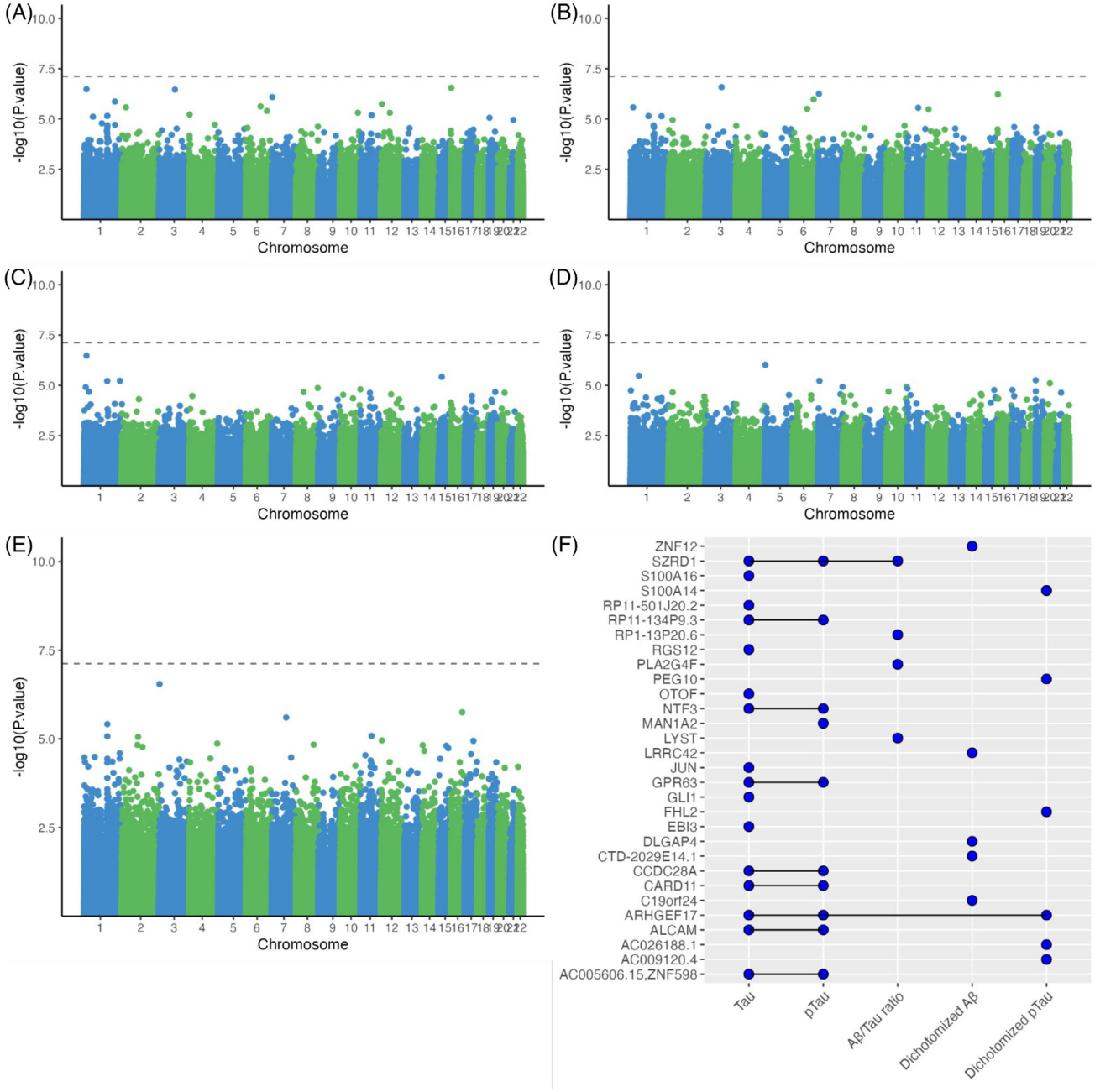


**FIGURE 1** EWAS of AD CSF biomarkers in 450 cognitively normal EHBS participants. Manhattan plots for the association between DNAm beta values and (A) tau, (B) p-tau, (C) Aβ/tau ratio, (D) Aβ±, (E) p-tau±. The dotted line represents the Bonferroni threshold ($p = 7.55 \times 10^{-8}$). F, UpSet plot showing overlapping associations across the five CSF biomarkers (t-tau, p-tau, Aβ42/t-tau, Aβ42±, p-tau ±). A blue dot represents an association between DNAm beta values and the corresponding CSF biomarker with a $p$ value $< 1 \times 10^{-5}$ for at least one CpG site assigned to the corresponding gene. All associations were adjusted for age at baseline, sex, smoking (with or without smoking history), and estimated cell-type proportions (B lymphocytes, natural killer cells, CD4 + T lymphocytes, CD8+ T lymphocytes, monocytes, neutrophils). Aβ, amyloid beta; AD, Alzheimer's disease; CSF, cerebrospinal fluid; DNAm, DNA methylation; EHBS, Emory Healthy Brain Study; EWAS, epigenome-wide association study; p-tau, phosphorylated tau; t-tau, total tau.

The distribution of Aβ42± was similar across the White EHBS participants and ADNI participants (EHBS White: 28.0% Aβ42+, ADNI: 31.1% Aβ42+) but the proportion of Black EHBS participants who were Aβ42+ was higher than both EHBS White participants and ADNI participants (48.9%) and similar to the PPMI participants (53.4%).

## 3.2 | Epigenome-wide association study

We investigated whether blood DNAm was associated with CSF Aβ42, p-tau, and t-tau biomarkers in 735 cognitively normal participants enrolled in EHBS, ADNI, and PPMI. In the EWAS of the White EHBS

participants ($N = 450$), no CpG sites were significant at the Bonferroni adjusted $p$ value $< 0.05$ threshold (equivalent to $p < 7.56 \times 10^{-8}$) for any of the AD CSF biomarkers after adjusting for age, sex, smoking history, and estimated cell-type proportions (B lymphocytes, natural killer cells, CD4 + T lymphocytes, CD8 + T lymphocytes, monocytes, neutrophils; Figure 1, Table 2, Figure S1 in supporting information). At a less stringent EWAS $p$ value threshold, we found several CpG sites with $p$ value $< 1 \times 10^{-5}$ for these CSF AD biomarkers and several were found in more than one AD biomarker with most of the overlap observed between t-tau and p-tau (Figure 1F). For example, cg03586820, which was closest to the gene *SZRD1*, was among the 10 most significant CpG sites for t-tau (effect estimate: −0.112, $p$ value: $3.31 \times 10^{-7}$), p-tau (effect estimate: −0.073, $p$ value: $2.64 \times 10^{-6}$) and Aβ42/t-tau (effect estimate: 0.003, $p$ value: $3.32 \times 10^{-7}$). The CpG site cg13422045, assigned to *ARHGEF17*, also showed similar associations across three CSF AD biomarkers (t-tau, p-tau, and p-tau ), with the strongest associations observed for p-tau (effect estimate: −0.010, $p$ value: $2.76 \times 10^{-6}$) and p-tau ± (effect estimate: −0.003, $p$ value: $8.23 \times 10^{-6}$; Figure 1F). None of the top 10 CpG sites with the smallest $p$ values for the association with the five CSF AD biomarkers could be replicated in ADNI or PPMI at a nominal $p$ value threshold of 5% (Table 2). Similarly, none of the top CpG sites could be replicated in the 45 Black/African American EHBS participants (for replication results, see Table S1 in supporting information).

We did not find any DMRs for any of the CSF AD biomarkers in EHBS.

## 3.3 | Epigenome-wide meta-analysis

In the EWAS meta-analysis of the White EHBS, ADNI, and PPMI participants ($N = 690$), one CpG site (cg17394795 [*RP11-53B5.1*] for Aβ42 ±) was significant at the Bonferroni-adjusted $p$ value $< 0.05$ threshold (equivalent to $p < 7.61 \times 10^{-8}$) after adjusting for age, sex, smoking history, and estimated cell-type proportions (Figure 2, Table 3, Figures S1–S4 in supporting information). However, there was substantial heterogeneity between the three cohorts ($I^2 = 74.6$, heterogeneity $p$ value = 0.019) and the association was no longer significant in the random-effects meta-analysis (Table S2 in supporting information; meta-analyzed $p$ value went from $1.84 \times 10^{-8}$ in the fixed-effects meta-analysis to 0.003 in the random-effects meta-analysis).

Among the top 10 CpG sites from the meta-analysis, five CpG sites were at least nominally significant (unadjusted $p$ value $< 0.05$) in all three cohorts, namely cg18609149 (*AC009950.2*) for p-tau, cg00679256 (*RP11-56I23.1*) and cg09606840 (*DNPH1*) for Aβ42+ vs. Aβ42−, and cg20708416 (*HOMER1*) and cg22663830 (*JUP*) for p-tau+ versus p-tau−. Among these, cg20708416 (*HOMER1*) showed the highest degree of heterogeneity across the cohorts ($I^2 = 42.3$), which led to a substantially larger $p$ value in the random effects meta-analysis ($p$ [fixed] $= 1.04 \times 10^{-5}$ versus $p$ [random] $= 0.001$).

Most associations were robust to additional adjustment for *APOE ε4* (Table S3 in supporting information). Of note, one of the top 10 CpG sites (cg22976567 [*LMNA*]) became genome-wide significant for t-tau after adjusting for *APOE ε4* (without $p = 1.39 \times 10^{-7}$ and with adjusting for *APOE ε4*: $p = 1.17 \times 10^{-8}$).

## 3.4 | Secondary analyses

To further aid the interpretation of our top associations, we performed a GO and KEGG pathway enrichment analysis based on the top 1000 CpG sites from the EHBS–EWAS and the meta-analyses with the lowest raw $p$ values. We did not identify any GO terms or KEGG pathways with an overrepresentation of genes containing significantly, differentially methylated CpGs that would indicate an enriched biological pathway. The top 10 GO terms and KEGG pathways that were nominally significant (raw $p < 0.05$) are included in the supporting information (see Tables S4–S7).

To evaluate the blood–brain concordance for DNAm beta values at our top 10 CpG sites, we used the BECon tool and Gene Expression Omnibus Database (accession code GSE111165). Several of the top 10 CpG sites from the EHBS–EWAS and the meta-analyses exhibited blood–brain concordance (Tables S8–S9 in supporting information). Among the top 10 CpG sites from the meta-analysis that were at least nominally significant in all three cohorts, cg09606840 (*DNPH1*, among the top 10 CpG sites for Aβ42 ± , mean brain–blood correlation = 0.246, 75th–90th percentile of positive mean brain–blood correlations according to BECon) exhibited blood–brain concordance according to BECon but not according to the Gene Expression Omnibus Database. The CpG site cg22663830 (*JUP*), which was among the top 10 CpGs for p-tau+ versus p-tau−, showed a significant brain–blood correlation according to the Gene Expression Omnibus Database (brain–blood correlation: 0.587, $p$ value: 0.006).

# 4 | DISCUSSION

In the present study, we conducted a blood EWAS of AD CSF biomarkers among 735 cognitively normal participants enrolled in the EHBS, ADNI, and PPMI cohorts. While this is one of the largest EWAS of AD CSF biomarkers and the first that was conducted among cognitively normal individuals, we found little evidence of an association between blood DNAm and AD CSF biomarkers in pre-clinical stages of AD. In the EHBS ($N = 450$ White participants), no CpG sites remained significant at the 5% Bonferroni threshold after adjusting for age, sex, smoking history, and estimated cell-type proportions. In the fixed-effects meta-analysis of the three cohorts, one CpG site (cg17394795 [*RP11-53B5.1*] for Aβ42 ±) was significant at the 5% Bonferroni threshold but the association was not confirmed in the random-effect meta-analysis. Another CpG site (cg22976567 [*LMNA*]) was significant for t-tau after additionally adjusting for *APOE ε4*. While not statistically significant, a few other CpG sites that were either among the top CpG sites for all three cohorts or showed a good agreement across several AD CSF biomarkers are noteworthy and should be further investigated in future studies.

**TABLE 2** Top 10 CpGs sites from the EWAS of AD CSF biomarkers in EHBS ($N = 450$ White participants) and their replication in ADNI ($N = 122$ White participants) and PPMI ($N = 118$ White participants).

| | | | EHBS | | ADNI | | PPMI | |
|---|---|---|---|---|---|---|---|---|
| CpG | Position | Gene | Effect estimate | *p* value | Effect estimate | *p* value | Effect estimate | *p* value |
| **A. t-tau** | | | | | | | | |
| cg25530374 | chr16:2047171 | AC005606.15, ZNF598 | −0.015 | 2.87e-07 | −0.035 | 0.160 | −0.001 | 0.923 |
| cg03586820 | chr1:16679780 | SZRD1 | −0.112 | 3.31e-07 | −0.002 | 0.993 | −0.071 | 0.239 |
| cg03376719 | chr3:105086940 | ALCAM | 0.012 | 3.49E-07 | −0.021 | 0.253 | 0.003 | 0.739 |
| cg19196826 | chr7:3018391 | CARD11 | 0.008 | 8.14e-07 | 0.003 | 0.846 | −0.002 | 0.720 |
| cg19769827 | chr1:203259772 | RP11-134P9.3 | 0.007 | 1.36e-06 | −0.013 | 0.541 | −0.002 | 0.879 |
| cg21719937 | chr12:5564478 | NTF3 | −0.013 | 1.80e-06 | 0.012 | 0.690 | 0.017 | 0.033 |
| cg09766383 | chr6:97285174 | GPR63 | 0.021 | 2.37e-06 | −0.018 | 0.605 | −9.31e-04 | 0.935 |
| cg16602332 | chr2:26735409 | OTOF | −0.021 | 2.64e-06 | 0.008 | 0.897 | 0.024 | 0.033 |
| cg06334093 | chr6:139094587 | CCDC28A | 0.013 | 4.00e-06 | 0.002 | 0.911 | 5.78e-04 | 0.931 |
| cg22546737 | chr10:118934495 | RP11-501J20.2 | 0.003 | 4.91e-06 | 0.009 | 0.171 | −0.001 | 0.685 |
| **B. p-tau** | | | | | | | | |
| cg03376719 | chr3:105086940 | ALCAM | 0.009 | 2.63e-07 | −0.008 | 0.202 | 1.34e-04 | 0.991 |
| cg19196826 | chr7:3018391 | CARD11 | 0.006 | 5.65e-07 | −7.39e-04 | 0.849 | 2.46e-04 | 0.965 |
| cg25530374 | chr16:2047171 | AC005606.15, ZNF598 | −0.010 | 5.99e-07 | −0.011 | 0.218 | 0.003 | 0.846 |
| cg06334093 | chr6:139094587 | CCDC28A | 0.009 | 1.05e-06 | −0.002 | 0.781 | 1.97e-04 | 0.984 |
| cg03586820 | chr1:16679780 | SZRD1 | −0.073 | 2.64e-06 | 0.014 | 0.825 | −0.068 | 0.353 |
| cg13422045 | chr11:73021272 | ARHGEF17 | −0.010 | 2.76e-06 | 0.014 | 0.240 | 0.024 | 0.160 |
| cg09766383 | chr6:97285174 | GPR63 | 0.015 | 3.13e-06 | −0.010 | 0.376 | −0.004 | 0.815 |
| cg21719937 | chr12:5564478 | NTF3 | −0.009 | 3.29e-06 | 0.008 | 0.401 | 0.023 | 0.032 |
| cg08186837 | chr1:117910444 | MAN1A2 | 0.003 | 7.08e-06 | −4.77e-04 | 0.888 | 0.006 | 0.445 |
| cg19769827 | chr1:203259772 | RP11-134P9.3 | 0.005 | 7.24e-06 | −0.007 | 0.287 | −0.004 | 0.761 |
| **C. Aβ42/t-tau** | | | | | | | | |
| cg03586820 | chr1:16679780 | SZRD1 | 0.003 | 3.32e-07 | 0.002 | 0.546 | −0.001 | 0.619 |
| cg10917153 | chr15:42448786 | PLA2G4F | 8.85e-04 | 3.78e-06 | 7.51e-04 | 0.644 | 8.39e-04 | 0.228 |
| cg13974715 | chr1:236009306 | LYST | 0.002 | 5.91e-06 | 1.61e-04 | 0.936 | −0.002 | 0.481 |
| cg09340250 | chr1:152924562 | RP1-13P20.6 | −0.003 | 6.07e-06 | −7.17e-05 | 0.987 | −9.08e-04 | 0.619 |
| cg11069276 | chr1:11718175 | FBXO44 | 9.10e-04 | 1.23e-05 | −0.002 | 0.102 | 7.25e-04 | 0.235 |
| cg10235683 | chr8:142304416 | SLC45A4 | 4.79e-04 | 1.36e-05 | −4.55e-04 | 0.519 | −3.70e-04 | 0.330 |
| cg00056692 | chr10:134947537 | GPR123 | 0.001 | 1.58e-05 | −0.002 | 0.193 | 0.001 | 0.083 |
| cg16158487 | chr1:33891592 | PHC2 | −6.78e-04 | 2.08e-05 | −6.41e-06 | 0.994 | 1.91e-04 | 0.740 |
| cg27030540 | chr19:41754975 | AXL | 0.001 | 2.15e-05 | −2.63e-04 | 0.830 | 3.63e-04 | 0.630 |
| cg05832751 | chr8:49716954 | EFCAB1 | 5.54e-04 | 2.16e-05 | −4.14e-04 | 0.630 | −5.23e-04 | 0.341 |
| **D. Aβ42+ versus Aβ42−** | | | | | | | | |
| cg08759359 | chr5:3288934 | CTD-2029E14.1 | 0.002 | 9.65e-07 | −0.002 | 0.047 | −0.003 | 0.354 |
| cg27047965 | chr1:54433172 | LRRC42 | 0.002 | 3.33e-06 | 5.01e-04 | 0.524 | −0.006 | 0.020 |
| cg15226147 | chr19:1275266 | C19orf24 | −0.014 | 5.55e-06 | 0.007 | 0.436 | −0.003 | 0.735 |
| cg27504433 | chr7:6741096 | ZNF12 | 0.002 | 5.96e-06 | 0.001 | 0.315 | 4.45e-04 | 0.863 |
| cg06769708 | chr20:35060706 | DLGAP4 | 0.001 | 7.90e-06 | 5.97e-04 | 0.415 | −0.003 | 0.260 |
| cg18890561 | chr10:131988419 | GLRX3 | −0.013 | 1.16e-05 | −0.011 | 0.093 | −0.008 | 0.164 |
| cg20673767 | chr7:158061805 | PTPRN2 | −0.024 | 1.19e-05 | 0.005 | 0.618 | 0.012 | 0.245 |
| cg20751395 | chr11:2594153 | KCNQ1 | 0.005 | 1.44e-05 | −3.26e-04 | 0.881 | −0.001 | 0.799 |

(Continues)

**TABLE 2** (Continued)

| CpG | Position | Gene | EHBS | | ADNI | | PPMI | |
|---|---|---|---|---|---|---|---|---|
| | | | Effect estimate | *p* value | Effect estimate | *p* value | Effect estimate | *p* value |
| cg16879549 | chr17:7146439 | CTD-2545G14.7 | −0.010 | 1.70e-05 | 0.007 | 0.407 | −2.07e-04 | 0.959 |
| cg02400458 | chr15:80624605 | LINC00927 | 0.002 | 1.71e-05 | 5.61e-04 | 0.530 | −0.004 | 0.044 |
| **E. p-tau+ versus p-tau−** | | | | | | | | |
| cg18254930 | chr3:3646624 | AC026188.1 | 0.009 | 2.85e-07 | −3.46e-04 | 0.921 | 0.002 | 0.816 |
| cg06763914 | chr16:74260395 | AC009120.4 | 0.061 | 1.78e-06 | −0.056 | 0.041 | 0.042 | 0.153 |
| cg11175683 | chr7:94286420 | PEG10 | −0.015 | 2.49e-06 | 0.002 | 0.796 | −1.09e-04 | 0.991 |
| cg16348003 | chr1:153589781 | S100A14 | 0.034 | 3.84e-06 | 0.012 | 0.503 | 0.005 | 0.748 |
| cg13422045 | chr11:73021272 | ARHGEF17 | −0.003 | 8.23e-06 | 0.002 | 0.165 | 0.003 | 0.303 |
| cg15104031 | chr1:153589528 | S100A14 | −0.014 | 8.43e-06 | −2.70e-04 | 0.963 | 0.007 | 0.414 |
| cg01928691 | chr2:106016014 | FHL2 | −0.036 | 8.78e-06 | −0.032 | 0.093 | −3.49e-04 | 0.982 |
| cg21719937 | chr12:5564478 | NTF3 | −0.003 | 1.11e-05 | 6.85e-04 | 0.634 | 0.006 | 0.002 |
| cg05734494 | chr17:57287309 | SMG8 | 0.002 | 1.14e-05 | −5.92e-04 | 0.441 | −0.002 | 0.353 |
| cg11537121 | chr4:184575108 | RWDD4 | 0.003 | 1.35e-05 | −2.80e-04 | 0.817 | −0.005 | 0.174 |

*Note*: All associations were adjusted for age at baseline, sex, smoking (except for PPMI), and estimated cell-type proportions. No CpG sites in EHBS were significant after adjusting for multiple testing (Bonferroni threshold: 7.55e-08).
Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; CSF, cerebrospinal fluid; EHBS, Emory Healthy Brain Study; PPMI, Parkinson's Progression Markers Initiative.

DNAm in cg22976567 (*LMNA*) was significantly associated with larger CSF t-tau values. The same CpG site was also among the top 20 CpGs associated with the Cardiovascular Risk Factors, Aging, and Dementia (CAIDE1) risk score in a meta-analysis of discovery and replication samples ($n \approx 400$ to $\approx 5000$) from Generation Scotland.[47] While interesting, the direction of association was different in the Generation Scotland study, in which DNAm in cg22976567 (*LMNA*) was associated with lower CAIDE1 risk scores, corresponding to a decreased risk of developing dementia. Increased expression of the *LMNA* gene has also been observed in the hippocampus of late-stage AD patients.[48] No other CpG sites were robustly associated with AD CSF biomarkers in the EWAS in EHBS or the meta-analysis across EHBS, ADNI, and PPMI. Our findings could have two potential explanations. First, the absence of a robust association between blood-based DNAm and AD CSF biomarkers in our study might indicate that epigenetic changes in the blood are not a good indicator of pre-clinical stages of AD. This hypothesis is further supported by the weak evidence for an association between blood-based DNAm and cognitive function among cognitively normal individuals. Specifically, a large-scale epigenome-wide meta-analysis of seven measures of cognitive functioning using data from 11 cohorts ($N = 6809$ healthy, older-aged adults) only identified two significant CpG associations with executive function and global cognitive ability.[49] Second, even when evaluating epigenetic signatures of AD CSF biomarkers among AD/MCI patients and controls, the most recent EWAS, which included 885 participants from the EMIF-AD study, also did not find strong evidence of an association for CSF amyloid measures and CSF tau variables, as no CpG sites passed the Bonferroni-significance threshold for those measures.[22] That study only identified associations between differential DNAm and CSF biomarkers of neuroinflammation (chitinase-3-like protein 1 [YKL-40]) and NfL. Another study of 202 ADNI participants identified several false discovery rate–significant loci associated with p-tau181 and Aβ42,[21] but none of those CpG sites could be replicated in the larger EMIF-AD study.[22]ptpt

While none of the CpG sites in the EHBS EWAS passed the Bonferroni-significance threshold, several CpG sites had *p* values $< 1 \times 10^{-5}$ for more than one CSF AD biomarker, with most of the overlap observed between t-tau and p-tau. For example, cg03586820 (*SZRD1*) was among the 10 most significant CpG sites for t-tau, p-tau, and Aβ42/t-tau, and cg13422045 (*ARHGEF17*) also showed similar associations across the tau-related CSF AD biomarkers (t-tau, p-tau, and p-tau ±). Interestingly, *ARHGEF17* has been associated with AD Braak stage,[50,51] schizophrenia,[52] and mortality[53] in previous studies.

A few CpG sites from our meta-analysis of EHBS, ADNI, and PPMI are noteworthy, as they were among the top 10 CpG sites in the epigenome-wide meta-analysis and at least nominally significant (unadjusted *p* value $< 0.05$) in all three cohorts. These include cg18609149 (*AC009950.2*), cg00679256 (*RP11-56I23.1*), cg09606840 (*DNPH1*), cg20708416 (*HOMER1*), and cg22663830 (*JUP*). Among these, *DNPH1*, *HOMER1*, and *JUP* have been previously reported in association with dementia-related outcomes.[54–56] *DNPH1* helps degrade cytotoxic nucleotides, preventing their incorporation into DNA, and one study reports that a variant associated with cognitive decline (rs45604140 [*PTK7*]) is predicted to affect *DNPH1* expression.[55] The *HOMER1* gene and the related protein Homer1a play a crucial role in the central nervous system and Homer1a is the best-known molecular marker of sleep need and has also been implicated in brain Aβ accumulation.[56] Finally, γ-catenin (*JUP*), is a

**TABLE 3** Top 10 CpGs sites from the epigenome-wide meta-analysis of AD CSF biomarkers in 450 cognitively normal individuals from the EHBS, 122 cognitively normal individuals from ADNI, and 118 cognitively normal individuals from PPMI.

| | | | Meta-analysis | | | | EHBS | | ADNI | | PPMI | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CpG | Position | Gene | Effect estimate | *p* value | $I^2$ | Het *p* value | Effect estimate | *p* value | Effect estimate | *p* value | Effect estimate | *p* value |
| **A. t-tau** | | | | | | | | | | | | |
| cg22976567 | chr1:156074182 | LMNA | 0.053 | 1.39e-07 | 0 | 0.670 | 0.047 | 1.01e-04 | 0.058 | 0.692 | 0.066 | 2.70e-04 |
| cg03586820 | chr1:16679780 | SZRD1 | −0.106 | 2.28e-07 | 0 | 0.716 | −0.112 | 3.31e-07 | −0.002 | 0.993 | −0.071 | 0.239 |
| cg25530374 | chr16:2047171 | AC005606.15, ZNF598 | −0.015 | 3.16e-07 | 7.9 | 0.338 | −0.015 | 2.87e-07 | −0.035 | 0.160 | −0.001 | 0.923 |
| cg03376719 | chr3:105086940 | ALCAM | 0.011 | 1.26e-06 | 48.6 | 0.143 | 0.012 | 3.49e-07 | −0.021 | 0.253 | 0.003 | 0.739 |
| cg20434604 | chr11:55416886 | OR4S2 | 0.188 | 1.43e-06 | 0 | 0.809 | 0.172 | 8.07e-04 | 0.458 | 0.443 | 0.209 | 5.77e-04 |
| cg05104523 | chr4:3295914 | RGS12 | −0.014 | 2.22e-06 | 28.8 | 0.245 | −0.014 | 6.00e-06 | −0.073 | 0.041 | −0.010 | 0.471 |
| cg19769827 | chr1:203259772 | RP11-134P9.3 | 0.007 | 2.42e-06 | 0 | 0.472 | 0.007 | 1.36e-06 | −0.013 | 0.541 | −0.002 | 0.879 |
| cg06598258 | chr1:21521902 | EIF4G3 | 0.039 | 3.62e-06 | 0 | 0.857 | 0.038 | 2.73e-04 | −0.013 | 0.905 | 0.043 | 0.004 |
| cg14086396 | chr5:140479263 | AC005754.7 | 0.150 | 4.35e-06 | 0 | 0.411 | 0.172 | 1.32e-04 | −0.198 | 0.496 | 0.135 | 0.005 |
| cg15926737 | chr12:57853737 | GLI1 | 0.009 | 5.64e-06 | 14.4 | 0.311 | 0.009 | 4.92e-06 | −0.021 | 0.293 | 0.012 | 0.324 |
| **B. p-tau** | | | | | | | | | | | | |
| cg25530374 | chr16:2047171 | AC005606.15, ZNF598 | −0.010 | 3.78e-07 | 0 | 0.716 | −0.010 | 5.99e-07 | −0.011 | 0.218 | 0.003 | 0.846 |
| cg22976567 | chr1:156074182 | LMNA | 0.037 | 2.53e-06 | 31 | 0.235 | 0.033 | 5.30e-05 | 0.022 | 0.650 | 0.083 | 0.004 |
| cg05104523 | chr4:3295914 | RGS12 | −0.010 | 2.82e-06 | 2.3 | 0.359 | −0.009 | 2.17e-05 | −0.025 | 0.021 | −0.014 | 0.420 |
| cg19196826 | chr7:3018391 | CARD11 | 0.005 | 3.13e-06 | 40 | 0.189 | 0.006 | 5.65e-07 | −7.39e-04 | 0.849 | 2.46e-04 | 0.965 |
| cg03586820 | chr1:16679780 | SZRD1 | −0.068 | 4.30e-06 | 0 | 0.397 | −0.073 | 2.64e-06 | 0.014 | 0.825 | −0.068 | 0.353 |
| cg03376719 | chr3:105086940 | ALCAM | 0.007 | 4.39e-06 | 71.6 | 0.030 | 0.009 | 2.63e-07 | −0.008 | 0.202 | 1.34e-04 | 0.991 |
| cg06334093 | chr6:139094587 | CCDC28A | 0.008 | 6.31e-06 | 43.1 | 0.172 | 0.009 | 1.05e-06 | −0.002 | 0.781 | 1.97e-04 | 0.984 |
| cg08186837 | chr1:117910444 | MAN1A2 | 0.003 | 1.06e-05 | 0 | 0.504 | 0.003 | 7.08e-06 | −4.77e-04 | 0.888 | 0.006 | 0.445 |
| cg13397720 | chr3:82682815 | RP11-260O18.1 | 0.113 | 1.11e-05 | 0 | 0.952 | 0.111 | 5.96e-05 | 0.092 | 0.523 | 0.134 | 0.090 |
| cg18609149 | chr2:230989183 | AC009950.2 | −0.060 | 1.30e-05 | 16.2 | 0.303 | −0.050 | 0.001 | −0.125 | 0.025 | −0.090 | 0.016 |
| **C. Aβ42/t-tau** | | | | | | | | | | | | |
| cg03586820 | chr1:16679780 | SZRD1 | 0.003 | 9.99e-07 | 26.9 | 0.255 | 0.003 | 3.32e-07 | 0.002 | 0.546 | −0.001 | 0.619 |
| cg10917153 | chr15:42448786 | PLA2G4F | 9.00e-04 | 1.59e-06 | 0 | 0.995 | 8.85e-04 | 3.78e-06 | 7.51e-04 | 0.644 | 8.39e-04 | 0.228 |
| cg05005308 | chr4:21305549 | KCNIP4 | −0.005 | 4.53e-06 | 39.7 | 0.190 | −0.005 | 3.37e-05 | 0.008 | 0.280 | −0.006 | 0.014 |
| cg13974715 | chr1:236009306 | LYST | 0.002 | 1.15e-05 | 0 | 0.410 | 0.002 | 5.91e-06 | 1.61e-04 | 0.936 | −0.002 | 0.481 |
| cg00056692 | chr10:134947537 | GPR123 | 0.001 | 1.36e-05 | 54.6 | 0.110 | 0.001 | 1.58e-05 | −0.002 | 0.193 | 0.001 | 0.083 |

(Continues)

**TABLE 3** (Continued)

| CpG | Position | Gene | Meta-analysis | | | | EHBS | | ADNI | | PPMI | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Effect estimate | *p* value | $I^2$ | Het *p* value | Effect estimate | *p* value | Effect estimate | *p* value | Effect estimate | *p* value |
| cg24716879 | chr1:235105872 | RP11-443B7.1 | 0.003 | 1.39e-05 | 9 | 0.333 | 0.002 | 5.17e-04 | 0.002 | 0.504 | 0.005 | 0.003 |
| cg09340250 | chr1:152924562 | RP1-13P20.6 | −0.003 | 1.65e-05 | 7.2 | 0.341 | −0.003 | 6.07e-06 | −7.17e-05 | 0.987 | −9.08e-04 | 0.619 |
| cg07311033 | chr2:111627862 | ACOXL | 0.001 | 1.92e-05 | 0 | 0.455 | 0.001 | 4.90e-05 | 0.002 | 0.077 | 3.71e-04 | 0.641 |
| cg18087266 | chr1:25257629 | RUNX3 | 0.001 | 2.59e-05 | 0 | 0.918 | 0.001 | 1.21e-04 | 3.90e-04 | 0.883 | 0.001 | 0.080 |
| cg14933468 | chr11:62138599 | ASRGL1 | −0.005 | 2.63e-05 | 0 | 0.594 | −0.005 | 2.38e-05 | −0.002 | 0.787 | −0.002 | 0.377 |
| **D. Aβ42+ versus Aβ42−** | | | | | | | | | | | | |
| **cg17394795** | **chr9:96628794** | **RP11-53B5.1** | **−0.011** | **1.84e-08** | **74.6** | **0.019** | **−0.007** | **0.002** | **−0.019** | **2.05e-06** | **−0.017** | **0.006** |
| cg08216368 | chr11:237063 | PSMD13 | −0.002 | 1.90e-07 | 0 | 0.581 | −0.002 | 7.22e-05 | −0.003 | 5.55e-04 | −0.002 | 0.460 |
| cg17173369 | chr6:169224099 | RP1-125N5.2 | −0.032 | 1.18e-06 | 34 | 0.220 | −0.028 | 0.001 | −0.012 | 0.544 | −0.049 | 7.96e-05 |
| cg18890561 | chr10:131988419 | GLRX3 | −0.012 | 1.22e-06 | 0 | 0.804 | −0.013 | 1.16e-05 | −0.011 | 0.093 | −0.008 | 0.164 |
| cg00679256 | chr10:107710219 | RP11-56I23.1 | −0.022 | 1.73e-06 | 0 | 0.441 | −0.019 | 8.87e-04 | −0.037 | 0.004 | −0.022 | 0.025 |
| cg09606840 | chr6:43197544 | DNPH1 | −0.016 | 2.44e-06 | 7.9 | 0.338 | −0.013 | 6.19e-04 | −0.022 | 0.008 | −0.029 | 0.019 |
| cg27504433 | chr7:6741096 | ZNF12 | 0.002 | 5.52e-06 | 0 | 0.640 | 0.002 | 5.96e-06 | 0.001 | 0.315 | 4.45e-04 | 0.863 |
| cg13589108 | chr1:177140680 | BRINP2 | 0.004 | 6.28e-06 | 0 | 0.802 | 0.004 | 4.75e-05 | 0.005 | 0.045 | 0.002 | 0.605 |
| cg12212774 | chr19:1615407 | TCF3 | 0.005 | 7.85e-06 | 41.5 | 0.181 | 0.005 | 1.96e-05 | 4.16e-04 | 0.894 | 0.010 | 0.023 |
| cg16676655 | chr6:33383412 | PHF1 | −0.003 | 8.08e-06 | 0 | 0.941 | −0.003 | 9.35e-05 | −0.003 | 0.073 | −0.005 | 0.211 |
| **E. p-tau+ versus p-tau−** | | | | | | | | | | | | |
| cg06598942 | chr4:139860154 | RP11-371F15.3 | 0.018 | 1.13e-06 | 73.5 | 0.023 | 0.019 | 3.59e-05 | −0.002 | 0.816 | 0.034 | 1.71e-04 |
| cg24716879 | chr1:235105872 | RP11-443B7.1 | −0.024 | 2.24e-06 | 0 | 0.469 | −0.025 | 2.54e-05 | −0.012 | 0.320 | −0.032 | 0.023 |
| cg18254930 | chr3:3646624 | AC026188.1 | 0.007 | 6.47e-06 | 67.1 | 0.048 | 0.009 | 2.85e-07 | −3.46e-04 | 0.921 | 0.002 | 0.816 |
| cg10935297 | chr14:35255491 | BAZ1A | 0.002 | 9.43e-06 | 0 | 0.604 | 0.002 | 2.18e-05 | 0.001 | 0.250 | 0.006 | 0.258 |
| cg21883293 | chr11:32334657 | RP1-65P5.1 | −0.007 | 9.78e-06 | 31.6 | 0.232 | −0.007 | 2.90e-05 | −0.002 | 0.571 | −0.013 | 0.031 |
| cg20708416 | chr5:78753093 | HOMER1 | 0.004 | 1.04e-05 | 42.3 | 0.177 | 0.003 | 0.004 | 0.006 | 0.004 | 0.009 | 0.012 |
| cg08490220 | chr16:2332752 | ABCA3 | 0.007 | 1.16e-05 | 0 | 0.844 | 0.007 | 1.57e-04 | 0.007 | 0.116 | 0.011 | 0.093 |
| cg22663830 | chr17:39928300 | JUP | 0.010 | 1.18e-05 | 0 | 0.525 | 0.009 | 0.001 | 0.011 | 0.043 | 0.017 | 0.015 |
| cg02671700 | chr8:64523255 | RN7SKP135 | 0.011 | 1.24e-05 | 0 | 0.580 | 0.012 | 1.21e-04 | 0.013 | 0.024 | 0.004 | 0.591 |
| cg12031108 | chr12:28115086 | PTHLH | 0.004 | 1.60e-05 | 70.4 | 0.034 | 0.003 | 0.011 | 0.008 | 1.80e-05 | −0.003 | 0.507 |

*Note*: All associations were adjusted for age at baseline, sex, smoking (except for PPMI), and estimated cell-type proportions. Bonferroni threshold: 7.61e-08. Associations that remained significant after adjusting for multiple testing are highlighted in **bold**.

Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; CSF, cerebrospinal fluid; EHBS, Emory Healthy Brain Study; PPMI, Parkinson's Progression Markers Initiative; p-tau, phosphorylated tau; t-tau, total tau.

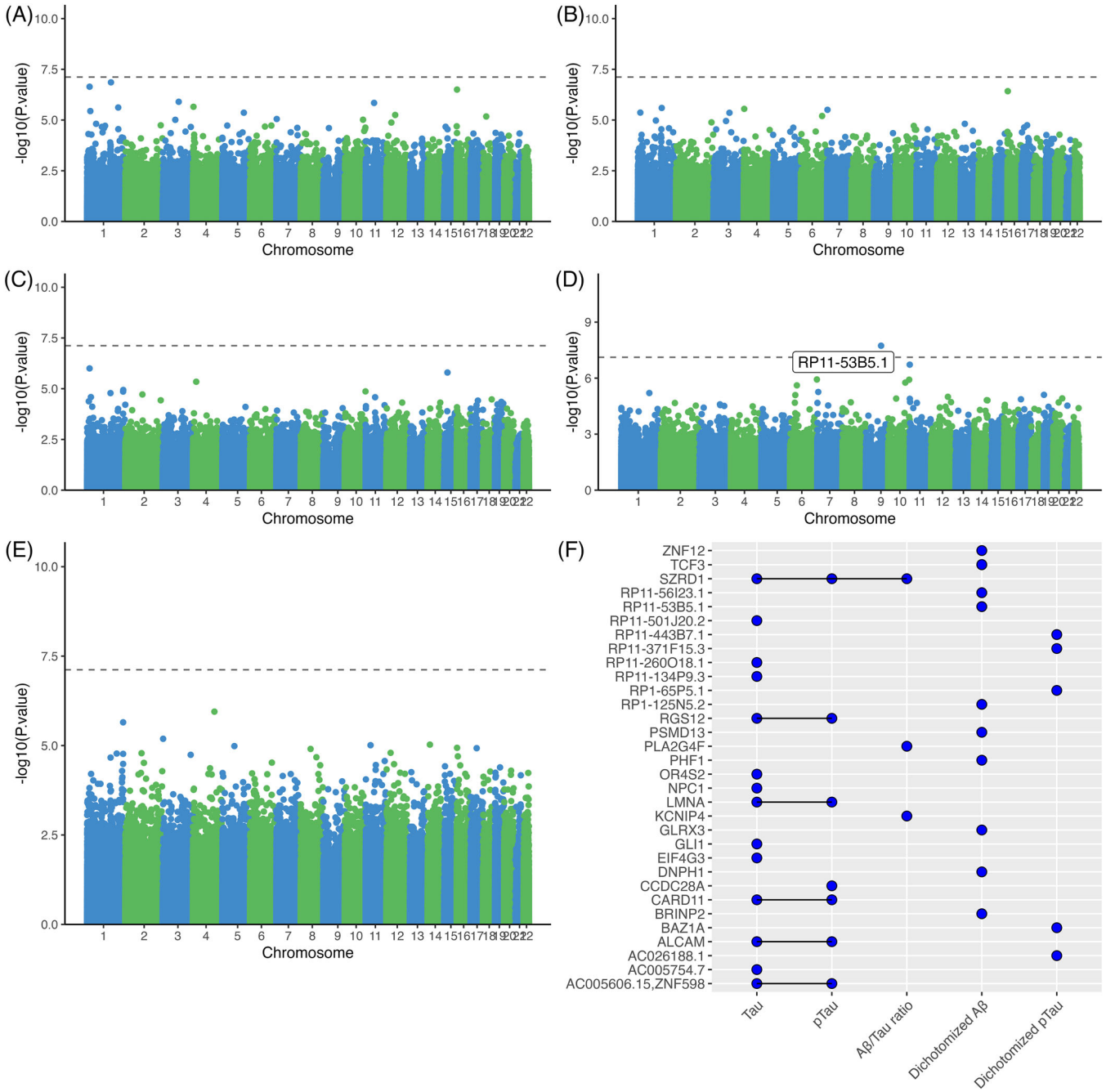


**FIGURE 2** Meta-analysis of CSF biomarkers EWAS of cognitively normal individuals from EHBS ($N = 450$), ADNI ($N = 122$), and PPMI ($N = 118$). Manhattan plots for the association between DNAm beta values and (A) tau, (B) p-tau, (C) Aβ/tau ratio, (D) Aβ ± , (E) p-tau ± . The dotted line represents the Bonferroni threshold ($p = 7.61 \times 10^{-8}$). F, UpSet plot showing overlapping associations across the five CSF biomarkers (t-tau, p-tau, Aβ42/t-tau, Aβ42 ± , p-tau ±). A blue dot represents an association between DNAm beta values and the corresponding CSF biomarker with a $p$ value $< 1 \times 10^{-5}$ for at least one CpG site assigned to the corresponding gene. Aβ, amyloid beta; AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; CSF, cerebrospinal fluid; DNAm, DNA methylation; EHBS, Emory Healthy Brain Study; EWAS, epigenome-wide association studies; PPMI, Parkinson's Progression Markers Initiative; p-tau, phosphorylated tau; t-tau, total tau.

crucial component of cell junctions, and is significantly upregulated in semantic dementia, a clinical subtype of frontotemporal dementia.[54]

Our study has several strengths. Most importantly, the EHBS is one of the largest prospective cohort studies with CSF samples from cognitively normal individuals. The level of depth in our outcome assessment, underscored by the inclusion of a substantial sample size with CSF measurements, a highly invasive and challenging-to-obtain biological fluid, provides a rare and valuable opportunity to understand potential associations between differential DNAm and AD CSF biomarkers among cognitively normal individuals. Furthermore,

our study is the first study of DNAm and AD CSF biomarkers that attempted a replication of findings in two independent cohorts, namely ADNI and PPMI, thus strengthening the robustness of our conclusions.

In addition to its strengths, our study has several limitations. First, the temporal sequence between differential DNAm and AD CSF biomarkers could not be clearly defined because both were assessed in blood samples collected at the same study visit. Furthermore, while all our study participants were cognitively normal, that is, without a diagnosis of cognitive impairment at blood draw, there were substantial differences between EHBS, ADNI, and PPMI participants related to the study design and the demographics. EHBS and PPMI participants were approximately 10 years younger than the ADNI participants and overall healthier. In addition, there were only 122 and 118 cognitively normal ADNI and PPMI participants with DNAm data, respectively (vs. 450 EHBS participants), which might have contributed to the lack of replication across the three cohorts. Another limitation of our study is the use of whole blood for DNAm profiling. Although we have tried to account for this by including cell proportions as covariates in our analyses, future research using DNA isolated from specific cell types would enable the identification of cell type–specific signatures related to the AD CSF biomarkers. Another limitation of our study is that we had to restrict our main analyses to White participants, and none of our findings could be replicated in our smaller population of Black/African American EHBS participants.

In conclusion, our EWAS of blood-based DNAm and AD CSF biomarkers among 735 cognitively normal participants enrolled in the EHBS, ADNI, and PPMI cohorts showed only weak evidence of an association between differential DNAm and AD CSF biomarkers assessed to evaluate pre-clinical stages of AD. Future studies should include additional biomarkers, for example, CSF biomarkers of neuroinflammation (YKL-40) and NfL or blood-based AD biomarkers, which might show a stronger correlation with blood-based DNAm.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests. Author disclosures are available in the supporting information.

## CONSENT STATEMENT

The Emory Healthy Brain Study was approved by the institutional review board of Emory University Medical Center. The Alzheimer's Disease Neuroimaging Initiative cohort and the Parkinson's Progression Markers Initiative cohort were approved by the institutional review board of each participating site. All participants provided written informed consent.

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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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