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Effect of Race on Prediction of Brain Amyloidosis by Plasma Aβ42/Aβ40, Phosphorylated Tau, and Neurofilament Light

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ABSTRACT

Objective: To evaluate whether plasma biomarkers of amyloid (Aβ42/Aβ40), tau (p-tau181 and p-tau231) and neuroaxonal injury (neurofilament light chain [NfL]) detect brain amyloidosis consistently across racial groups.

Methods: Individuals enrolled in studies of memory and aging who self-identified as African American (AA) were matched 1:1 to self-identified non-Hispanic White (NHW) individuals by age, *APOE* ε4 carrier status and cognitive status. Each participant underwent blood and cerebrospinal fluid (CSF) collection, and amyloid PET was performed in 103 participants (68%). Plasma Aβ42/Aβ40 was measured by a high-performance immunoprecipitation-mass

spectrometry assay. Plasma p-tau181, p-tau231, and NfL were measured by Simoa immunoassays. CSF Aβ42/Aβ40 and amyloid PET status were used as primary and secondary reference standards of brain amyloidosis, respectively.

Results: There were 76 matched pairs of AA and NHW participants (n=152 total). For both AA and NHW groups, the median age was 68.4 years, 42% were *APOE* ε4 carriers and 91% were cognitively normal. AA were less likely than NHW to have brain amyloidosis by CSF Aβ42/Aβ40 (22% versus 43% positive, $p = 0.003$). The Receiver Operating Characteristic Area Under the Curve (ROC AUC) of CSF Aβ42/Aβ40 status with the plasma biomarkers was as follows: Aβ42/Aβ40, 0.86 (95% confidence intervals [CI] 0.79-0.92); p-tau181, 0.76 (0.68-0.84); p-tau231, 0.69 (0.60-0.78); and NfL, 0.64 (0.55-0.73). In models predicting CSF Aβ42/Aβ40 status with plasma Aβ42/Aβ40 that included covariates (age, sex, *APOE* ε4 carrier status, race, and cognitive status), race did not affect the probability of CSF Aβ42/Aβ40 positivity. In similar models based on plasma p-tau181, p-tau231 or Nfl, AA had a lower probability of CSF Aβ42/Aβ40 positivity (Odds Ratio [OR] 0.31 [95% CI 0.13-0.73], OR 0.30 [0.13-0.71]) and OR 0.27 [0.12-0.64], respectively. Models of amyloid PET status yielded similar findings.

Conclusions: Models predicting brain amyloidosis using a high performance plasma Aβ42/Aβ40 assay may provide an accurate and consistent measure of brain amyloidosis across AA and NHW groups, but models based on plasma p-tau181, p-tau231, and NfL may perform inconsistently and could result in disproportionate misdiagnosis of AA.

Keywords

Alzheimer disease, race, biomarker, blood, plasma, amyloidosis

Introduction

Biomarkers of Alzheimer disease (AD) brain pathology are used by research studies, clinical trials, and memory clinics for a variety of indications, including to determine whether the etiology of cognitive impairment is likely to be related to AD or another cause. Amyloid positron emission tomography (PET) is a well-established technique to determine whether an individual has significant brain amyloidosis that could be causing or contributing to cognitive impairment; however, amyloid PET is expensive and has limited availability [1]. Cerebrospinal fluid (CSF) biomarkers are also highly accurate predictors of brain amyloidosis and are less expensive, but skilled clinicians are required to perform lumbar puncture (LP) procedures, and some individuals perceive LPs as invasive [2]. Several commercial assays can be used to measure concentrations of CSF amyloid-β peptide 42 (Aβ42), Aβ40, total tau (t-tau), and tau phosphorylated at position 181 (p-tau181), and cut-offs consistent with brain amyloidosis have been established [3-5].

Notably, biomarker cut-offs for brain amyloidosis have been defined in cohorts comprised largely of non-Hispanic White (NHW) individuals, and then applied to all individuals. However, several studies have found lower levels of CSF t-tau and p-tau181 in African Americans (AA) as compared to NHW, even after adjusting for factors such as age, sex, *APOE* ε4 carrier status, and cognitive impairment [6-9]. Why AA have lower levels of CSF t-tau and p-tau181 is unknown and could be due to differences in medical comorbidities, biological factors, or social determinants of health [8, 10, 11]. Regardless of the underlying reasons, these differences have important implications for the utility of CSF biomarkers. Applying biomarker cut-offs defined in NHW to groups in which the biomarker has not been studied could potentially subject the other groups to additional testing, incorrect medical management, missed opportunities for treatment with AD-specific therapies, and lower enrollment in AD clinical trials [9, 12]. However, it

is also highly problematic to "adjust" the interpretation of medical tests based on race, especially given the heterogeneity represented within racial groups and dynamic nature of race because it is a social rather than a biological construct [9, 13, 14]. Rather, it would be much preferable to use AD biomarkers that perform accurately and consistently across racial and ethnic groups. Alternatively, adjusting for the factors that underly racial differences in AD biomarkers (e.g., medical comorbidities) may be more valid and generalizable across groups.

Over the last three years there has been rapid development of blood-based biomarkers for AD [15]. The PrecivityAD™ test offered by C2N Diagnostics, which includes highly precise measurement of plasma Aβ42/Aβ40 and apolipoprotein E (apoE) proteotype by mass spectrometry, is now available for clinical use [16, 17]. Multiple plasma p-tau isoforms can also be used as biomarkers of brain amyloidosis, including p-tau181 [18, 19], p-tau217 [20-22] and p-tau231 [23]. Plasma neurofilament light chain (NfL) may also be useful as a non-specific marker of neuroaxonal injury [24]. It is critical to evaluate whether these assays accurately and consistently predict brain amyloidosis across various racial and ethnic groups. In this study, one of the largest cohorts of AA with CSF biomarker and amyloid PET information was used to examine the relationship of these reference measures of brain amyloidosis with the C2N Diagnostics PrecivityAD assay for plasma Aβ42/Aβ40 as well as Simoa immunoassays for p-tau181, p-tau231, and NfL.

Methods

Participants

This study analyzed samples and data from the Charles F. and Joanne Knight Alzheimer Disease Research Center (ADRC), which includes one of the largest groups of AA in AD

research who have undergone CSF collection and/or amyloid PET. The cohort consists of community-dwelling older adults recruited from the St. Louis area, including participants with and without cognitive impairment, who enrolled in research studies of memory and aging at Washington University in St. Louis. Participants underwent clinical and cognitive assessments using the Uniform Data Set (UDS) [25] that includes the Clinical Dementia Rating® (CDR®) [26] and Mini-Mental State Examination (MMSE) [27]. The UDS includes the Hollingshead two factor index of social position, which assigns a social class based on the participant's educational level and the occupation of the head of the participant's household [28]. Presence or absence of hypertension or diabetes was noted by the clinician. Race and gender were self-identified.

Participants with CSF biomarker information and adequate aliquots of plasma available for analysis were considered for inclusion. Each self-identified AA participant was matched 1:1 to a self-identified NHW participant by a computer algorithm. Participants were matched by age at the time of plasma collection (within two years), *APOE* ε4 status (carrier or non-carrier) and cognitive status at the time of plasma collection (cognitively normal [CDR=0] or cognitively impaired [CDR>0]). If more than one NHW participant matched an AA participant, the participant with the closest age was selected.

Standard Protocol Approvals, Registrations, and Patient Consents

Written informed consent was obtained from all participants and their study partners. All procedures were approved by Washington University's Human Research Protection Office.

Genotyping

The *APOE* genotype was determined by genotyping rs7412 and rs429358 with Taqman genotyping technology[29]. Genetic sex determined by sex-chromosome specific analysis was concordant with gender in all individuals in this cohort.

CSF and Plasma Collection and Analysis

CSF and blood samples from each participant were collected at a single session at approximately 8 am following overnight fasting as previously described [5, 30]. Concentrations of CSF Aβ40, Aβ42, total tau (t-tau), and tau phosphorylated at 181 (p-tau181) were measured by chemiluminescent enzyme immunoassay using a fully automated platform (LUMIPULSE G1200, Fujirebio, Malvern, PA, USA). CSF NfL was measured via commercial ELISA kit (UMAN Diagnostics, Umeå, Sweden). Plasma Aβ42 and Aβ40 were measured in the C2N Diagnostics commercial laboratory with an immunoprecipitation-mass spectrometry assay (St. Louis, MO, USA) [16]. Plasma p-tau181 and p-tau231 were measured in the Clinical Neurochemistry Laboratory, University of Gothenburg (Mölndal, Sweden) using in-house Single molecule array (Simoa) assays on an HD-X analyzer (Quanterix, Billerica, MA, USA), as previously described [19, 23]. Plasma NfL was measured with Quanterix Nf-Light assay kits at Washington University (St. Louis, Missouri, USA) on a HD-X analyzer. All assays were performed by personnel who were blind to participant information.

Amyloid PET

Participants underwent a dynamic scan with either Florbetapir (n=48) or Pittsburgh Compound B (PiB, n=55) in coordination with a structural MRI scan. Regional data from the 30-60 minute post-injection window for PiB and the 50-70 minute window for Florbetapir was converted to standardized uptake value ratios (SUVRs) using cerebellar grey as a reference and partial volume corrected using a geometric transfer matrix approach based upon the Freesurfer parcellation [31]. Values from regions where amyloid deposition occurs early in AD were averaged together to represent mean cortical SUVR, which was converted to centiloid using previously published equations [32, 33].

Statistical Analysis

The significance of differences by self-identified race were evaluated with Wilcoxon ranked sum tests for continuous variables and Chi-Square or Fisher exact tests for categorical variables. The covariate-adjusted significance of racial differences were evaluated using ANCOVA models with biomarker concentrations as the outcome measure, self-identified race as the predictor variable, and including the covariates of age, sex, *APOE* ε4 carrier status and cognitive status (cognitively normal [CDR=0] or cognitively impaired [CDR>0]). Models used natural logarithm transformed values for CSF and plasma p-tau181 and NfL, which were positively skewed. Models including the interaction between race and *APOE* ε4 carrier status were also evaluated.

CSF Aβ42/Aβ40 status was chosen as the primary reference standard for brain amyloidosis because all individuals in the study had both CSF and blood collected at the same session, whereas only a sub-cohort had an amyloid PET scan performed within two years of

CSF/blood collection. Positive CSF Aβ42/Aβ40 was defined by a CSF Aβ42/Aβ40 < 0.0673, a cut-off that maximally distinguished amyloid PET status in an overlapping cohort with a Receiver Operating Characteristic Area Under the Curve (ROC AUC) of 0.97 [34]. Amyloid PET positivity was previously defined as a mean cortical SUVR > 1.42 for PiB and > 1.19 for Florbetapir [32, 35]. Logistic regression models were implemented with CSF Aβ42/Aβ40 or amyloid PET status as the outcome measure and each plasma biomarker as the predictor variable. Covariate adjusted models included self-identified race, sex, age, *APOE* ε4 carrier status, and cognitive status. Models that additionally included either the interaction between race and *APOE* ε4 carrier status or race and plasma biomarker levels were evaluated. Differences between ROC AUCs were evaluated using the DeLong test [36].

Statistical analyses were implemented using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Plots were created with GraphPad Prism version 9.2.0 (GraphPad Software, La Jolla, CA, USA). All *p* values were from two-sided tests, and results were deemed statistically significant at $p < 0.05$.

Data Availability Policy

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

Results

Participant characteristics

Based on the inclusion criteria of CSF biomarker information and adequate aliquots of plasma available for analysis, 79 AA and 775 NHW participants were potentially eligible for the

study. Each AA participant was matched 1:1 to a NHW participant by age, *APOE* ε4 carrier status and cognitive status. Three AA participants who could not be matched to a NHW participant were not included in the study. The final study cohort included a total of 152 participants (76 AA and 76 matching NHW) who contributed samples that underwent measurement of plasma biomarkers (see **Table 1** for cohort characteristics). An amyloid PET scan was performed within two years of plasma collection in 49 AA (64%) and 54 NHW (71%) participants (**eTable 1**). All AA participants identified their ethnicity as non-Hispanic.

For both the AA and NHW groups, the median age was 68.4 years old, 42% carried at least one *APOE* ε4 allele (8% were ε4 homozygotes), and 9% were cognitively impaired as defined by a CDR>0. There was no difference in dementia severity by race as measured by the CDR. Both the AA and NHW groups were well-educated (median of 16 years of education), but the AA group had a slightly lower social position than the NHW group as measured by the Hollingshead two factor index of social position (median 2.0 [interquartile range (IQR) 2.0-3.5] versus 2.0 [IQR 1.0-3.0], respectively; $p < 0.002$). Since the AA and NHW participants had no significant differences in years of education, this suggests that the median occupational level of the head of household in the AA group was lower (e.g., fewer of the AA participants lived in households headed by executives/major professionals). Compared to NHW, AA were more likely to have hypertension (67% versus 45%, $p = 0.006$) or diabetes (28% versus 5%, $p = 0.0003$).

CSF and plasma biomarkers by race

CSF Aβ42 and Aβ40 concentrations were not significantly different between the AA and NHW groups (**Table 1**). However, AA had higher CSF Aβ42/Aβ40 (median 0.0874 [IQR 0.0681

to 0.0935] versus 0.0719 [0.0477 to 0.0870], $p < 0.0001$) and lower amyloid PET centiloid (median 2.3 [IQR -1.0 to 10.1] versus 10.1 [0.0-33.0], $p = 0.02$), consistent with the AA group having lower average levels of brain amyloidosis compared to the NHW group (**Figure 1**). In the overall cohort, 22% of the AA and 43% of the NHW groups had brain amyloidosis by CSF Aβ42/Aβ40 status ($p = 0.003$); in the sub-cohort with amyloid PET, 10% of AA and 39% of the NHW groups had brain amyloidosis by amyloid PET status ($p = 0.003$). Plasma Aβ42 was only slightly higher in the AA group ($p = 0.03$) and plasma Aβ40 did not vary by racial group, but plasma Aβ42/Aβ40 was markedly higher in the AA group (median 0.1047 [IQR 0.0990-0.1101] versus 0.0963 [0.0904-0.1028], $p < 0.0001$), again consistent with the AA group having lower average levels of brain amyloidosis compared to the NHW group. CSF total tau and p-tau181 were lower in the AA group than the NHW group ($p = 0.002$ and $p = 0.0008$, respectively), but there were no statistically significant differences in plasma p-tau181 and p-tau231 between racial groups. There was a trend towards lower CSF NfL in AA compared to NHW ($p = 0.08$), but there was no difference in plasma NfL by racial group.

Plasma biomarkers, CSF Aβ42/Aβ40 or amyloid PET centiloid, and race

Nonlinear associations between plasma biomarkers and CSF Aβ42/Aβ40 or amyloid PET centiloid were examined by Spearman correlations as depicted in **Figures 2-3** and **eFigures 1-2** and summarized in **eTable 2**. Of the plasma biomarkers, Aβ42/Aβ40 had the strongest correlations with CSF Aβ42/Aβ40 ($\rho = 0.52$ [0.39 to 0.63]) and amyloid PET centiloid (-0.30 [-0.10 to -0.47]) after adjustment for covariates. To examine the relationships between the plasma biomarkers, brain amyloid, and race, biomarker concentrations were modeled as a function of CSF Aβ42/Aβ40 status and included race, age, sex, *APOE* ε4 carrier status and cognitive status

as covariates (**Table 2**). More abnormal (lower) plasma Aβ42/Aβ40 were associated with NHW race ($p < 0.0001$), male sex ($p < 0.0001$), and positive CSF Aβ42/Aβ40 status ($p < 0.0001$). In contrast, more abnormal (higher) plasma p-tau181 levels were associated with older age ($p < 0.0001$), positive CSF Aβ42/Aβ40 status ($p = 0.003$), male sex ($p = 0.01$), and impaired cognitive status ($p = 0.02$). More abnormal (higher) p-tau231 levels were associated with impaired cognitive status ($p = 0.0009$), older age ($p = 0.01$) and positive CSF Aβ42/Aβ40 status ($p = 0.03$). More abnormal (higher) plasma NfL levels were associated with older age ($p < 0.0001$) and impaired cognitive status ($p = 0.03$). Similar models of plasma biomarker levels including amyloid PET status rather than CSF Aβ42/Aβ40 status yielded similar results except that cognitive status was not a significant predictor in any model (**eTables 3-6**); few participants with cognitive impairment had amyloid PET data (4 of 103), limiting power to detect differences by cognitive status in these models. Models that additionally included the interaction between race and *APOE* ε4 carrier status were evaluated, but the interaction was not significant for any model and therefore it was not included in the final analyses.

Correspondence of plasma biomarkers with CSF Aβ42/Aβ40 and amyloid PET status

Prediction of CSF Aβ42/Aβ40 or amyloid PET status by plasma biomarkers was evaluated by logistic regression analyses as depicted in **Figures 2-3** and **eFigures 1-2**, shown in **eTables 7-11,** and summarized in **Tables 3** and **4.** Models predicting CSF Aβ42/Aβ40 status based on plasma biomarker levels had ROC AUCs as follows: Aβ42/Aβ40, 0.86 (95% confidence intervals [CI] 0.79-0.92); p-tau181, 0.76 (0.68-0.84); p-tau231, 0.69 (0.60-0.78); and NfL, 0.64 (0.55-0.73). The amyloid probability score, a proprietary modeled value provided by

C2N Diagnostics that is based on plasma Aβ42/Aβ40, apoE proteotype and age [17], had a ROC AUC of 0.89 (0.84-0.95) with CSF Aβ42/Aβ40 status. Comparisons of ROC AUCs showed that plasma Aβ42/Aβ40 had significantly better prediction of CSF Aβ42/Aβ40 status compared to p-tau181, p-tau231 and NfL ($p < 0.05$, 0.004, and <0.0001, respectively, **Table 3**).

Covariate adjusted models of CSF Aβ42/Aβ40 status incorporating each plasma biomarker and covariates (age, sex, *APOE* ε4 carrier status, race and cognitive status) are summarized in **Table 4**. The model based on plasma Aβ42/Aβ40 had a ROC AUC of 0.90 (0.85-0.96) (**eTable 7)**, which was superior to a model of covariates alone (0.82 [0.74-0.89] (**eTable 12**), $p = 0.006$ for difference in ROC AUCs). In the model of CSF Aβ42/Aβ40 status incorporating plasma Aβ42/Aβ40 and covariates, a higher probability of CSF Aβ42/Aβ40 positivity was associated with *APOE* ε4 carriers (odds ratio [OR] 5.6 [95% CI 2.0-16], $p = 0.001$), older age in years (OR 1.12 [1.03-1.21], $p = 0.007$), and cognitive impairment (OR 9.2 [1.9-46], $p = 0.007$). Notably, in models incorporating plasma Aβ42/Aβ40 and covariates, race did not significantly affect correspondence with CSF Aβ42/Aβ40 or amyloid PET status.

The covariate adjusted model for CSF Aβ42/Aβ40 status based on p-tau181 had a ROC AUC of 0.85 (0.79-0.92) (**Table 4, eTable 9**). In this model, a higher probability of CSF Aβ42/Aβ40 positivity was associated with *APOE* ε4 carriers (OR 5.7 [2.3-14], $p = 0.0002$), cognitive impairment (OR 7.7 [1.7-36], $p = 0.009$), and older age in years (OR 1.08 [1.00-1.15], $p = 0.04$), while AA race was associated with a lower probability of positivity (OR 0.31 [0.13-0.73], $p = 0.007$). Models of CSF Aβ42/Aβ40 or amyloid PET status based on p-tau231 (**eTable 10)** or NfL (**eTable 11**) were also evaluated and summarized in **Table 4**.

A model of CSF Aβ42/Aβ40 status based only on covariates demonstrates that AA race was associated with a lower probability of CSF Aβ42/Aβ40 positivity (OR 0.27 [0.12-0.64], $p =$

0.003) (**eTable 12**). Importantly, AA race significantly decreased the probability of CSF Aβ42/Aβ40 positivity in models based on plasma p-tau181 (OR 0.31 [0.13-0.73], $p = 0.007$), p-tau231 (OR 0.30 [0.13-0.71], $p = 0.006$) or NfL (OR 0.27 [0.12-0.64], $p = 0.003$) levels. Consistent with these results, AA race decreased the probability of amyloid PET positivity in models including plasma p-tau181 (OR 0.19 [0.06-0.63], $p = 0.007$), p-tau231 (OR 0.17 [0.05-0.59], $p = 0.005$) or NfL (OR 0.17 [0.05-0.55], $p = 0.003$) levels (**eTables 9, 10, and 11,** respectively). In contrast, race did not affect the probability of CSF Aβ42/Aβ40 or amyloid PET positivity associated with plasma Aβ42/Aβ40 (**eTable 7**). Models of CSF Aβ42/Aβ40 status including only cognitively normal individuals (91% of cohort) showed the same major findings as models that included the entire cohort (**eTable 13).** Models of CSF Aβ42/Aβ40 status were also evaluated that incorporated either the interaction between race and *APOE* ε4 carrier status or race and plasma biomarker levels, but neither interaction was significant for any model and therefore the interactions were not included in the final analyses.

Combining plasma biomarkers

A model of CSF Aβ42/Aβ40 status including levels of all plasma biomarkers and covariates had a ROC AUC of 0.92 (0.88-0.96), which was not significantly different from the ROC AUC of the model including Aβ42/Aβ40 as the only plasma biomarker (**eTable 14**). In the model with all plasma biomarkers, plasma Aβ42/Aβ40 was the only biomarker that was a significant predictor ($p < 0.0001$): plasma p-tau181, p-tau231 and NfL were not significant predictors of CSF Aβ42/Aβ40 after adjusting for the effects of plasma Aβ42/Aβ40 and covariates. In a similar model of amyloid PET status, plasma Aβ42/Aβ40 and plasma NfL levels were both significant predictors ($p = 0.0004$ and $p = 0.007$, respectively). In models of CSF

Aβ42/Aβ40 or amyloid PET status with all plasma biomarkers and covariates (including plasma Aβ42/Aβ40), race was not a significant predictor.

Discussion

This study found that the C2N Diagnostics PrecivityAD plasma Aβ42/Aβ40 assay more accurately classified CSF Aβ42/Aβ40 or amyloid PET status, as compared to Simoa-based assays for plasma p-tau181, p-tau231 and NfL, in a mostly cognitively normal cohort of matched AA and NHW research participants. Self-identified race did not affect prediction of CSF Aβ42/Aβ40 or amyloid PET status by plasma Aβ42/Aβ40. However, AA had a significantly lower probability of CSF or amyloid PET positivity compared to NHW in models incorporating plasma p-tau181, p-tau231, or NfL levels, suggesting that predictive algorithms for these assays would perform inconsistently across racial groups and that applying cut-offs established in NHW to AA could lead to disproportionate misdiagnosis of AA.

Plasma biomarkers have been almost exclusively studied in non-Hispanic White cohorts, with little data available on the performance of these biomarkers in other groups. A recent study of a multiracial cohort found good performance of plasma p-tau217 in distinguishing clinical, pathological, and amyloid PET status, but performance of the assay in predicting amyloid PET status across racial groups could not be ascertained because only forty individuals had amyloid PET data [37]. Another study found that plasma p-tau181 and plasma p-tau181/Aβ42 were associated with brain amyloidosis and hippocampal atrophy in a Singaporean AD cohort with high burden of cerebrovascular disease, but it did not investigate potential plasma biomarker differences across racial groups [38]. Plasma NfL has been studied in a large Latino cohort, but amyloid PET data was only available in a relatively small subset of participants [39]. To reduce racial disparities in research and clinical care, it is important to confirm that plasma biomarker

assays have accurate and consistent performance in identifying amyloid status across racial and ethnic groups.

Comparing the absolute values of biomarkers corrected for covariates may be misleading in evaluating which biomarkers perform consistently across racial groups. For example, in this study AA had higher average plasma Aβ42/Aβ40 compared to NHW, but this reflected lower levels of brain amyloidosis in AA and did not affect the probability of CSF Aβ42/Aβ40 positivity associated with a given plasma Aβ42/Aβ40 value. In contrast, plasma p-tau181 levels did not vary by race, but AA were less likely to be amyloid positive at a given plasma p-tau181 value. Without a comparison to reference standards, investigators might have concluded that plasma Aβ42/Aβ40 was more variable across racial groups and that p-tau isoforms were more consistent, when in fact plasma Aβ42/Aβ40 was accurately detecting differences in brain amyloidosis by racial group. Confirming that plasma biomarker assays have accurate and consistent performance in identifying amyloid status across racial and ethnic groups requires comparison with a reference standard, and not just covariate-adjusted models of absolute levels.

Previous studies have found an inconsistent relationship between amyloid biomarkers and race. One study found that AA had higher measures of amyloid PET [40] while another recent study found the opposite result [12]. Some studies have found no differences in CSF Aβ42 levels by racial group [6-8], but the current findings demonstrate that CSF Aβ42 alone may miss significant racial differences that are apparent when CSF Aβ42/Aβ40 is evaluated. The inconsistent relationship between race and amyloid biomarkers could reflect variation in recruitment methods: NHW and AA are often recruited differently (e.g., NHW are more often referred by healthcare providers and AA are more often referred by community contacts) [41, 42]. Recruitment differences could result in racial groups having significantly different comorbidities,

social determinants of health, or frequencies of brain amyloidosis. Potential differences in brain amyloidosis by racial group again suggest that comparison of plasma biomarkers with a reference standard, rather than comparison of absolute values, may be more helpful in establishing which plasma biomarker assays are accurate and consistent across racial groups.

One important issue in the fluid biomarker field is that different assays for plasma analytes have widely varying performance. A recent head-to-head comparison of eight different plasma Aβ42/Aβ40 assays found ROC AUCs with CSF Aβ42/Aβ40 status ranging from a maximum of 0.86 for the Washington University assay that is the basis for the C2N assay used in this study down to a minimum of 0.69 for some immunoassays (0.50 is chance alone) [43]. In another head-to-head comparison study, different p-tau assays yielded somewhat different findings, even for the same p-tau isoform [44]. The differences in assay performance complicate comparisons of the relationship of different biomarker analytes to factors such as race. For example, it is unclear whether the probability of CSF Aβ42/Aβ40 or amyloid PET positivity would be affected by race in models incorporating plasma p-tau181, p-tau231 or p-tau217 measured with higher performing assays (e.g. ROC AUC of > 0.85 with CSF Aβ42/Aβ40 and/or amyloid PET status). Additionally, performance of plasma assays may vary markedly in prediction of brain amyloidosis depending on the study cohort. For example, the p-tau181 assay used in the current study performed very well in predicting amyloid PET status in a cohort including both cognitively normal and cognitively impaired individuals (ROC AUC 0.88) [19], but the performance was lower when predicting amyloid PET status in cognitively normal individuals (ROC AUC 0.82) [45]. Overall, use of consistently high-performing assays is needed to make accurate conclusions about comparative associations of biomarkers.

Although this study made use of one of the largest AD research cohorts with CSF and amyloid PET data, there are major limitations in the conclusions. Individuals enrolled in this study were primarily from the greater St. Louis metropolitan area and individuals from other geographic regions may vary in key characteristics such as medical comorbidities or social determinants of health. The very small number of individuals with cognitive impairment (7 of 76 in each group) was not sufficient to allow analysis of the relationships between cognitive impairment, race, and biomarker levels. This study of 76 matched pairs of individuals, in which six variables had significant effects, was also not sufficiently powered to evaluate the underlying reasons for the racial differences. The Hollingshead index of social position demonstrated that AA had a slightly lower social position compared to NHW. However, this measure does not capture the complex social factors that may underlie biomarker differences between the groups. Further, AA had a higher rate of hypertension and diabetes compared to NHW, but the relatively small cohort did not permit a detailed investigation of these effects. For example, only four NHW had diabetes, which does not permit analysis of race by diabetes interactions. Although this study is insufficiently powered or does not have the data available to answer many important questions, it does document racial differences in plasma biomarkers that could potentially lead to clinical misdiagnosis, bias clinical trials that use a biomarker cut-off for inclusion [12, 46], and impact interpretation of biomarkers as a secondary endpoint. These findings should further encourage investigators to evaluate the performance of plasma biomarker assays in diverse cohorts. Further, this report strengthens the justification for the creation of large, diverse cohorts that are adequately powered to evaluate the underlying reasons for racial differences.

It is critical to understand that biomarker differences associated with race likely reflect differences in medical comorbidities, social determinants of health, and/or the effects of systemic

racism, rather than inherent biological differences [10]. For example, in this study cohort there were differences in the rates of hypertension and diabetes by racial group, and recent work has demonstrated that major medical comorbidities such as heart and kidney disease may affect plasma biomarker levels [47]. AD research cohorts have traditionally not collected detailed information about social determinants of health such as economic stability, access to healthy foods, neighborhood safety, and quality of education that may be associated with dementia; the importance of these factors is now gaining greater recognition [48]. Fortunately, the greater accessibility and acceptance of blood-based AD biomarkers may enable creation of larger cohorts and increased inclusion of groups, such as AA, that have been under-represented in AD biomarker studies [49]. Much larger longitudinal studies of diverse cohorts are needed to evaluate the intersection of race, AD biomarkers, cognitive impairment, medical comorbidities, and social determinants of health [50]. Improved understanding of these complex factors will enable more accurate AD diagnosis and improve patient care for all groups.

Appendix 1: Authors

Name	Location	Contribution
Suzanne E. Schindler, MD, PhD	Washington University	Design and conceptualization of study; major role in the acquisition of data; analyzed the data; drafted the manuscript for intellectual content
Thomas K. Karikari, PhD	University of Gothenburg	Major role in the acquisition of data; interpreted the data; revised the manuscript for intellectual content
Nicholas J. Ashton, PhD	University of Gothenburg	Major role in the acquisition of data; interpreted the data; revised the manuscript for intellectual content
Rachel L. Henson, MS	Washington University	Major role in the acquisition of data; interpreted the data; revised the manuscript for intellectual content
Kevin E. Yarasheski, PhD	C2N Diagnostics	Major role in the acquisition of data; interpreted the data; revised the manuscript for intellectual content
Tim West, PhD	C2N Diagnostics	Major role in the acquisition of data; interpreted the data; revised the manuscript for intellectual content
Matthew R. Meyer, PhD	C2N Diagnostics	Major role in the acquisition of data; interpreted the data; revised the manuscript for intellectual content
Kristopher M. Kirmess, PhD	C2N Diagnostics	Major role in the acquisition of data; interpreted the data; revised the manuscript for intellectual content
Yan Li, PhD	Washington University	Analyzed the data; revised the manuscript for intellectual content
Benjamin Saef, MS	Washington University	Analyzed the data; revised the manuscript for intellectual content
Krista L. Moulder, PhD	Washington University	Interpreted the data; revised the manuscript for intellectual

		content
David Bradford	Washington University	Interpreted the data; revised the manuscript for intellectual content
Anne M. Fagan, PhD	Washington University	Interpreted the data; revised the manuscript for intellectual content
Brian A. Gordon, PhD	Washington University	Interpreted the data and recommended additional analyses; revised the manuscript for intellectual content
Tammie L.S. Benzinger, MD, PhD	Washington University	Major role in the acquisition of data; interpreted the data; revised the manuscript for intellectual content
Joyce Balls-Berry, PhD	Washington University	Interpreted the data; revised the manuscript for intellectual content
Randall J. Bateman, MD	Washington University	Interpreted the data and recommended additional analyses; revised the manuscript for intellectual content
Chengjie Xiong, PhD	Washington University	Analyzed the data; revised the manuscript for intellectual content
Henrik Zetterberg, MD, PhD	University of Gothenburg	Major role in the acquisition of data; interpreted the data; revised the manuscript for intellectual content
Kaj Blennow, MD, PhD	University of Gothenburg	Major role in the acquisition of data; interpreted the data; revised the manuscript for intellectual content
John C. Morris, MD	Washington University	Major role in the acquisition of data; interpreted the data; revised the manuscript for intellectual content

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Table 1. Characteristics of Knight Alzheimer Disease Research Center matched cohort. Continuous values are presented as the median with the interquartile range. The significance of differences by self-identified race were evaluated with Wilcoxon ranked sum tests for continuous variables and Chi-Square or Fisher exact tests for categorical variables. The covariate-adjusted significance of racial differences was evaluated using ANCOVA models with biomarker concentrations at the outcome measure, race as the predictor variable, and the covariates of age, sex, *APOE* ε4 carrier status and cognitive status. Plasma p-tau181 and NfL were transformed with the natural logarithm in covariate-adjusted models.

Characteristic	African American Participants n = 76		Non-Hispanic White Participants n = 76		*p* =	Adjusted *p* =
Demographics						
Age at CSF collection (years)	68.4 (64.9-73.2)		68.4 (64.1-73.1)		N.S.	
Sex (n, % Female)	44, 58%		39, 51%		N.S.	
APOE ε4 status (n, % carrier)	32, 42%		32, 42%		N.S.	
CDR 0/0.5/1 (% >0)	69/4/3 (9%)		69/5/2 (9%)		N.S.	
Years of education	16 (12-18)		16 (14-18)		N.S.	
Hollingshead index	2.0 (2.0-3.5)		2.0 (1.0-3.0)		0.002	
Hypertension (yes/no/not reported, % yes of reported)	51/25/0 (67%)		33/40/3 (45%)		0.006	
Diabetes (yes/no/not reported, % yes of reported)	21/55/0 (28%)		4/69/3 (5%)		0.0003	
CSF/plasma to LP interval (years)	0.11 (0.05-0.21)		0.08 (0.04-0.23)		N.S.	
CSF biomarker concentrations						
CSF Aβ42 (pg/ml)	76	735 (544-971)	76	682 (516-883)	N.S.	N.S.
CSF Aβ40 (pg/ml)	76	9490 (7150-11600)	76	10100 (8880-12300)	0.07	N.S.
CSF Aβ42/Aβ40	76	0.0874 (0.0681-0.0935)	76	0.0719 (0.0477-0.0870)	0.0003	0.0001
CSF Aβ42/Aβ40 <0.0673 (n, %)	76	17, 22%	76	33, 43%	0.006	0.003
CSF total tau (pg/ml)	76	212 (165-287)	76	290 (217-482)	0.0002	0.002
CSF p-tau181 (pg/ml)	76	31 (24.6-41.1)	76	38.0 (30.4-55.7)	0.002	0.0008
CSF NfL (pg/mL)	72	644 (493-868)	76	736 (542-973)	0.09	0.08
Plasma biomarker concentrations						
Plasma Aβ42 (pg/ml)	76	41.9 (39.3-49.6)	76	40.9 (37.8-46.3)	0.06	0.03
Plasma Aβ40 (pg/ml)	76	409 (380-470)	76	425 (390-482)	N.S.	N.S.
Plasma Aβ42/Aβ40	76	0.1047 (0.0990-0.1101)	76	0.0963 (0.0904-0.1028)	<0.0001	<0.0001
Plasma p-tau181 (pg/ml)	76	12.3 (10.2-16.2)	76	14.2 (10.6-19.3)	N.S.	N.S.
Plasma p-tau231 (pg/ml)	76	8.2 (4.4-11.3)	76	9.1 (6.6-13.1)	0.09	N.S.
Plasma NfL (pg/ml)	76	11.1 (7.6-15.5)	76	11.8 (8.9-16.7)	N.S.	N.S.
Amyloid PET						
Amyloid PET centiloid	49	2.3 (-1.0-10.1)	54	10.1 (0.0-33.0)	0.01	0.02
Amyloid PET positive	49	5, 10%	54	21, 39%	0.0008	0.003

Table 2. Relationship between plasma biomarkers, CSF Aβ42/Aβ40 status and covariates. Analysis of covariance models evaluated the effects of CSF Aβ42/Aβ40 status (positive < 0.0673), self-identified race, sex, age, *APOE* ε4 carrier status and cognitive status on levels of each plasma biomarker. Plasma p-tau181 and NfL were transformed with the natural logarithm for analysis.

Plasma Aβ42/Aβ40			
Parameter	**Estimate**	**S.E.**	***p* =**
Intercept	0.1052	0.0051	<0.0001
CSF Aβ42/Aβ40 status (positive)	-0.008	0.0013	<0.0001
Race (African American)	0.0060	0.0011	<0.0001
Sex (female)	0.0044	0.0011	<0.0001
Age (years)	-0.00010	0.00007	N.S.
APOE ε4 status (carrier)	-0.0009	0.0011	N.S.
Cognitive status (CDR>0)	-0.0010	0.0019	N.S.

Ln (plasma p-tau181)			
Parameter	**Estimate**	**S.E.**	***p* =**
Intercept	1.267	0.311	<0.0001
CSF Aβ42/Aβ40 status (positive)	0.239	0.079	0.003
Race (African American)	-0.044	0.066	N.S.
Sex (female)	-0.164	0.065	0.01
Age (years)	0.020	0.004	<0.0001
APOE ε4 status (carrier)	0.017	0.068	N.S.
Cognitive status (CDR>0)	0.278	0.115	0.02

Plasma p-tau231			
Parameter	**Estimate**	**S.E.**	***p* =**
Intercept	-1.655	4.474	N.S.
CSF Aβ42/Aβ40 status (positive)	2.525	1.140	0.03
Race (African American)	-0.970	0.946	N.S.
Sex (female)	-0.985	0.940	N.S.
Age (years)	0.160	0.063	0.01
APOE ε4 status (carrier)	-0.190	0.985	N.S.
Cognitive status (CDR>0)	5.585	1.651	0.0009

Ln (plasma NfL)			
Parameter	**Estimate**	**S.E.**	***p* =**
Intercept	-0.710	0.357	0.05
CSF Aβ42/Aβ40 status (positive)	-0.015	0.091	N.S.
Race (African American)	-0.091	0.075	N.S.
Sex (female)	-0.052	0.075	N.S.
Age (years)	0.046	0.005	<0.0001
APOE ε4 status (carrier)	0.092	0.079	N.S.
Cognitive status (CDR>0)	0.297	0.132	0.03

Table 3. CSF Aβ42/Aβ40 or amyloid PET status as predicted by plasma Aβ42/Aβ40 and covariates. Logistic regression models evaluated prediction of CSF Aβ42/Aβ40 (positive < 0.0673) or amyloid PET status by each plasma biomarker alone (unadjusted models) or plasma biomarkers and the covariates of self-identified race, sex, age, *APOE* ε4 carrier status and cognitive status (adjusted models). The amyloid probability score is a proprietary modeled value that incorporates plasma Aβ42/Aβ40, age and apolipoprotein E proteotype. Plasma p-tau181 and NfL were transformed with the natural logarithm for analysis. For each model, the Receiver Operating Characteristic Area Under the Curve (ROC AUC) with 95% confidence intervals is shown. The significance of each biomarker as a predictor in the model (biomarker *p* =) and the difference between the ROC AUC for the plasma Aβ42/Aβ40 model and other models (versus plasma Aβ42/Aβ40 *p*=) is shown.

Prediction of CSF Aβ42/Aβ40 status (n=152)

	Unadjusted model			Covariate adjusted model		
	ROC AUC	**Biomarker *p* =**	**Versus plasma Aβ42/Aβ40 *p* =**	**ROC AUC**	**Biomarker *p* =**	**Versus plasma Aβ42/Aβ40 *p* =**
Plasma Aβ42/Aβ40	0.86 (0.79-0.92)	<0.0001	reference	0.90 (0.85-0.96)	<0.0001	reference
Amyloid probability score	0.89 (0.84-0.95)	<0.0001	0.05	0.91 (0.87-0.96)	<0.0001	N.S.
Ln (plasma p-tau181)	0.76 (0.68-0.84)	<0.0001	<0.05	0.85 (0.79-0.92)	0.007	N.S.
Plasma p-tau231 (pg/ml)	0.69 (0.60-0.78)	0.0002	0.004	0.85 (0.78-0.91)	0.01	0.07
Ln (plasma NfL)	0.64 (0.55-0.73)	0.008	<.0001	0.81 (0.74-0.89)	N.S.	0.005
Covariates alone	N.A.	N.A.	N.A.	0.82 (0.74-0.89)	N.A.	0.006

Prediction of amyloid PET status (n=103)

	Unadjusted model			Covariate adjusted model		
	ROC AUC	**Biomarker *p* =**	**Versus plasma Aβ42/Aβ40 *p* =**	**ROC AUC**	**Biomarker *p* =**	**Versus plasma Aβ42/Aβ40 *p* =**
Plasma Aβ42/Aβ40	0.86 (0.77-0.95)	<0.0001	reference	0.89 (0.82-0.97)	0.0004	reference
Amyloid probability score	0.90 (0.82-0.97)	<0.0001	N.S.	0.90 (0.84-0.96)	0.0006	N.S.
Ln (plasma p-tau181)	0.74 (0.63-0.84)	0.002	0.05	0.84 (0.75-0.92)	0.02	N.S.
Plasma p-tau231 (pg/ml)	0.69 (0.58-0.81)	0.004	0.02	0.84 (0.75-0.92)	0.01	N.S.
Ln (plasma NfL)	0.55 (0.43-0.67)	N.S.	<0.0001	0.82 (0.73-0.91)	N.S.	N.S.
Covariates alone	N.A.	N.A.	N.A.	0.81 (0.72-0.90)	N.A.	0.08

Table 4. CSF Aβ42/Aβ40 status as predicted by plasma biomarkers and covariates. Logistic regression models evaluated prediction of CSF Aβ42/Aβ40 status (positive < 0.0673) by each plasma biomarker and the covariates of self-identified race, sex, age, *APOE* ε4 carrier status and cognitive status. Plasma p-tau181 and NfL were transformed with the natural logarithm for analysis. For each model, the Receiver Operating Characteristic Area Under the Curve (ROC AUC) with 95% confidence intervals is shown.

Plasma Aβ42/Aβ40, ROC AUC 0.90 (0.85-0.96)			
Parameter	**Estimate**	**SE**	***p* =**
Intercept	13.0	4.7	0.005
Plasma Aβ42/Aβ40 (pg/ml)	-220	46	<0.0001
Race (African American)	0.058	0.274	N.S.
Sex (female)	0.843	0.568	N.S.
Age (years)	0.109	0.04	0.007
APOE ε4 status (carrier)	0.865	0.269	0.001
Cognitive status (CDR>0)	1.11	0.41	0.007

Plasma p-tau181, ROC AUC 0.85 (0.79-0.92)			
Parameter	**Estimate**	**SE**	***p* =**
Intercept	-8.69	2.71	0.001
Ln (plasma p-tau181)	1.53	0.57	0.007
Race (African American)	-0.59	0.22	0.007
Sex (female)	-0.21	0.44	N.S.
Age (years)	0.072	0.035	0.04
APOE ε4 status (carrier)	0.87	0.23	0.0002
Cognitive status (CDR>0)	1.02	0.39	0.009

Plasma p-tau231, ROC AUC 0.85 (0.78-0.91)			
Parameter	**Estimate**	**SE**	***p* =**
Intercept	-6.95	2.50	0.006
Plasma p-tau231 (pg/ml)	0.098	0.040	0.01
Race (African American)	-0.60	0.22	0.006
Sex (female)	-0.37	0.43	N.S.
Age (years)	0.096	0.034	0.004
APOE ε4 status (carrier)	0.94	0.23	<0.0001
Cognitive status (CDR>0)	1.07	0.38	0.006

Plasma NfL, ROC AUC 0.81 (0.74-0.89)			
Parameter	**Estimate**	**SE**	***p* =**
Intercept	-6.20	2.41	0.01
Ln (plasma NfL)	-0.097	0.476	N.S.
Race (African American)	-0.65	0.22	0.003
Sex (female)	-0.50	0.42	N.S.
Age (years)	0.109	0.040	0.007
APOE ε4 status (carrier)	0.89	0.23	<0.0001
Cognitive status (CDR>0)	1.27	0.39	0.001

Figure Legends

Figure 1. Biomarkers by race. Biomarkers of amyloid (**A**) tau (**B**) and neuroaxonal injury (**C**) are shown by self-identified race. The covariate-adjusted significance of racial differences were evaluated using ANCOVA models with biomarker concentrations at the outcome measure, race as the predictor variable, and the covariates of sex, age, *APOE* ε4 carrier status and cognitive status. Plasma p-tau181 and NfL were transformed with the natural logarithm for analysis. Point types denote the following: 1) race: red, AA; black, NHW; 2) cognitive status: open circle, CDR 0; closed square, CDR>0.

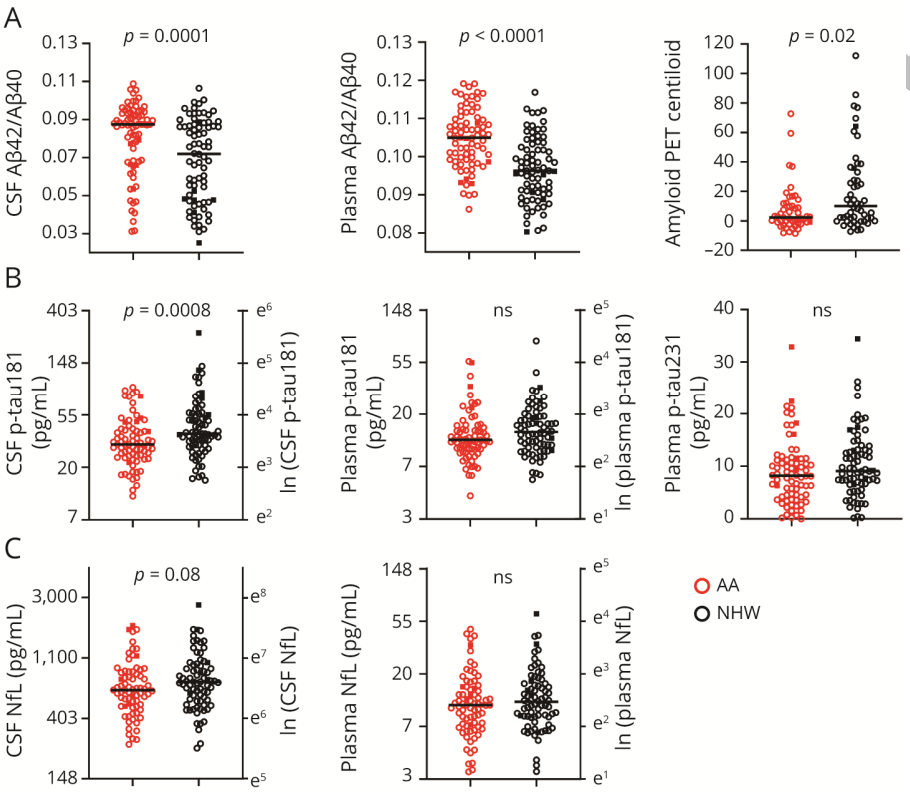


Figure 2. Relationship of plasma Aβ42/Aβ40 with CSF Aβ42/Aβ40 and amyloid PET. The relationship between plasma Aβ42/Aβ40 and CSF Aβ42/Aβ40 (**A**) or amyloid PET centiloid (**C**) was evaluated by partial Spearman correlation and was adjusted for age, sex, *APOE* ε4 carrier status, self-identified race, and cognitive status. Vertical dotted lines represent cut-off values for amyloid positivity. Plasma Aβ42/Aβ40 for AA and NHW groups were evaluated by CSF Aβ42/Aβ40 status (positive < 0.0673) (**B**) or amyloid PET status (**D**). Cut-off values for plasma Aβ42/Aβ40 with the highest combined sensitivity and specificity for distinguishing amyloid status were selected and are denoted by horizontal dashed lines. The Receiver Operating Characteristic Area Under the Curve (ROC AUC), positive percent agreement (PPA) and negative percent agreement (NPA) are shown. Point types denote the following: 1) race: red, AA; black, NHW; 2) cognitive status: open circle, CDR 0; closed square, CDR>0.

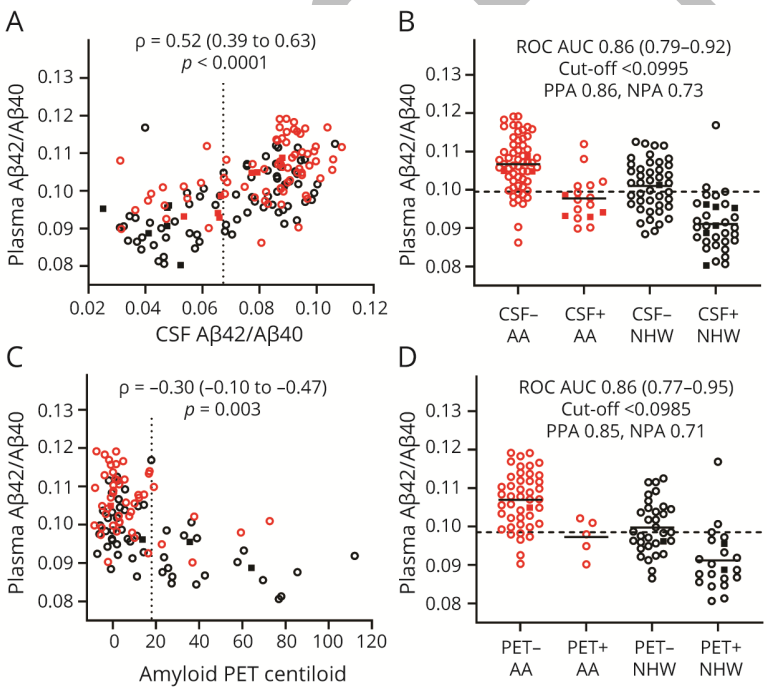
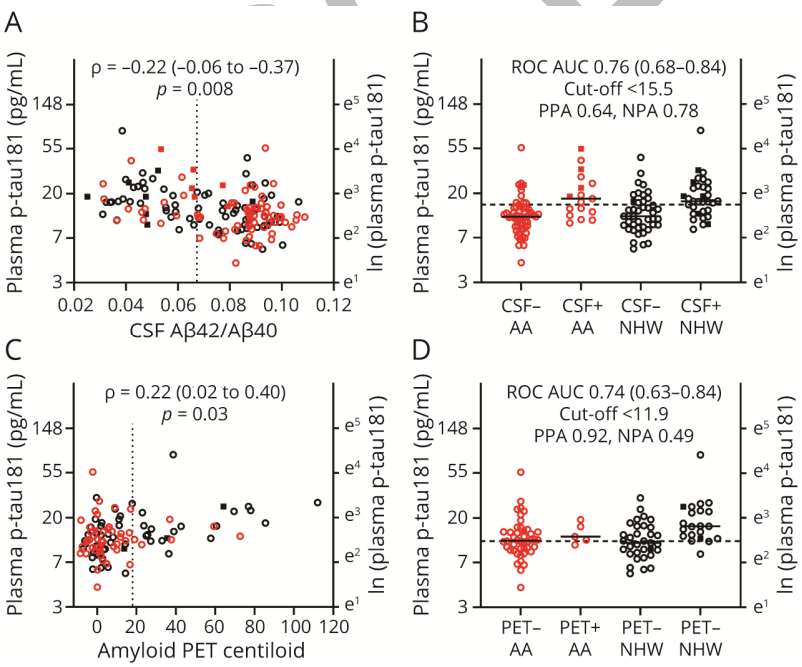


Figure 3. Relationship of plasma p-tau181 with CSF Aβ42/Aβ40 and amyloid PET. Plasma p-tau181 was transformed with the natural logarithm for analysis. The relationship between plasma p-tau181 and CSF Aβ42/Aβ40 (**A**) or amyloid PET centiloid (**C**) was evaluated by partial Spearman correlation and was adjusted for age, sex, *APOE* ε4 carrier status, self-identified race, and cognitive status. Vertical dotted lines represent cut-off values for amyloid positivity. Plasma p-tau181 levels for AA and NHW groups were evaluated by CSF Aβ42/Aβ40 status (positive < 0.0673) (**B**) or amyloid PET status (**D**). Cut-off values for plasma p-tau181 with the highest combined sensitivity and specificity for distinguishing amyloid status were selected and are denoted by horizontal dashed lines. The Receiver Operating Characteristic Area Under the Curve (ROC AUC), positive percent agreement (PPA) and negative percent agreement (NPA) are shown. Point types denote the following: 1) race: red, AA; black, NHW; 2) cognitive status: open circle, CDR 0; closed square, CDR>0.



Effect of Race on Prediction of Brain Amyloidosis by Plasma Aβ42/Aβ40, Phosphorylated Tau, and Neurofilament Light

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