ARIC Manuscript Proposal #4235

PC Reviewed: 5/09/23	**Status: _____**	**Priority:2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1a. Full Title: Predictors of change in blood-based biomarkers of Alzheimer's disease pathology and neurodegeneration measured from mid- to late-life and their associations with late-life measures of brain health in the Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters): plasma AD biomarkers and brain health

2. Writing Group:

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I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. **(please confirm with your initials electronically or in writing] X**

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3. Timeline: Submit manuscript within 3-6 months of proposal approval. Submit abstract on preliminary analyses of self-reported physical activity and blood biomarkers to AAIC (due April 24th, 2023) to reference in an NIH progress report update.

4. Rationale:

Decades of advances in Alzheimer's disease (AD) research, particularly in cerebrospinal fluid (CSF) and brain imaging biomarkers,[1,2] have led to the dominance of 3 neuropathological constructs: brain amyloid beta, brain neurofibrillary tangles comprised of phosphorylated tau, and neurodegeneration. Current understanding of the natural history leading to dementia due to AD can be summarized as follows:[1] the 2 main proteinopathies underlying AD, amyloid and tau, are separate processes, and amyloid deposition can accelerate tau deposition; amyloid and tau

deposition precede and cause neurodegeneration, which leads to the clinical syndromes of mild cognitive impairment (MCI) and dementia. The constructs of amyloid, tau, and neurodegeneration feature prominently in the recent National Institute on Aging (NIA)/Alzheimer's Association (AA) 2018 research framework.[3] This framework proposes to conduct research in which individuals are classified by the presence or absence of evidence of amyloid (A), tau (T), and neurodegeneration (N), with or without clinical manifestations, for the purpose of better understanding the mechanisms and sequence of neuropathology. The 2018 research framework has been enabled by the widespread availability of accurate CSF and brain imaging markers of amyloid, tau, and neurodegeneration (AT(N)).

Biomarkers in CSF and brain imaging, however, are expensive and not practical for use in large epidemiologic studies. Novel plasma-based biomarkers of AD neuropathology are less burdensome and might be more cost-effective and feasible compared to these current gold standard methods. Traditionally, blood-based biomarkers for AD have had barriers precluding their clinical use including issues with lower limit of detection, depletion of lower molecular weight proteins, and antibody availability that have limited their use.[4] However, several advancements have been made in the measurement of blood-based biomarkers. More recently, ultrasensitive immunoassays coupled with mass spectrometry show greater promise.[5] In particular, the commercially available Single-Molecule Array (Simoa™) is a novel method to measure Aβ40, Aβ42, phosphorylated tau, and neurofilament light (NfL) in plasma.[6]

Due to the commercial availability of ultrasensitive immunoassays over the last decade, there has been an exponential increase in studies measuring blood-based biomarkers of AD pathology and assessing their prognostic value in predicting clinical cognitive outcomes.[7] However, few studies have examined changes in the blood biomarkers as potential markers of disease progression, particularly during the mid- to late-life transition period, in a community-based population. The largest prospective analysis of blood biomarkers to date was conducted in the Rotterdam Study[8] of 4,444 participants (mean age: 71.9 years, 57.5% female) where trajectories of Aβ40, Aβ42, total tau and NfL were compared among individuals with Alzheimer's disease (AD) dementia compared to those who remained dementia-free throughout follow-up. Plasma NfL increased linearly for all participants, but the annual rate of change was 3.4 times faster among individuals with baseline AD dementia compared to those without. Aβ42 declined over time in participants with AD dementia, but the rate of change was not significantly different from those without dementia. A recent study in the Alzheimer's Disease Neuroimaging Initiative (ADNI)[9] examined if baseline and longitudinal plasma p-tau 181 was associated with AD-specific neurodegeneration. In a sample of 1,113 participants (mean age: 74 years, 46.1% female, 89.1% non-Hispanic White) from ADNI, longitudinal increases in both p-tau 181 and NfL were associated with decreases in gray matter volume and cognitive decline. These associations were evident in both participants with cognitive impairment and participants without cognitive impairment, suggesting that plasma p-tau 181 may indicate pre-symptomatic disease changes and therefore enable assessing disease progression earlier in the course of decline. Similarly, among 250 participants (mean age: 70 years, 32% female) in the Swedish BioFINDER Study[10], increase in phosphorylated tau-217 over 6 years was correlated with greater decline in cognition and more brain atrophy. Limitations of these prior studies include homogenous study samples precluding generalizability to non-White populations and analyses restricted to older adults where pathologic changes may be more advanced.

Therefore, more data are needed to understand whether temporal changes in the biomarkers are associated with structural brain morphology, AD-related pathology, and clinical

cognitive outcomes of interest, but also to characterize predictors of changes in blood biomarkers over the lifecourse. There is great need to consider the constructs of amyloid, tau, and neurodegeneration from the 2018 NIA//AA research framework within the context of longitudinal cohort studies—particularly community- and population-based cohorts to inform our understanding of the temporal relationships and patterns of the biomarkers in relation to disease progression. We have a unique opportunity within the ARIC cohort to contribute to this growing body of literature, and to be among the first studies to examine longitudinal changes in blood-based biomarkers during the mid- to late-life transition period when pathological changes in the brain are most likely to begin.

5. Main Hypothesis/Study Questions:

Aim 1 (Descriptive Models): Examine cross-sectional and longitudinal associations of demographic, behavioral, biological, and comorbidity risk factors with blood biomarker levels of AD pathology and neurodegeneration and change in blood biomarkers over 20 years in an informative biracial community-based sample of adults.

Aim 1a (cross-sectional): Examine cross-sectional associations of demographic (sex, race, APOE-e4, education), behavioral, biological, and comorbidity measures with plasma p-tau 181, Aβ42/Aβ40 ratio, NfL and GFAP at Visit 3 (n=1840, 1993-1995, mean age: 52 years) and Visit 5 (n=1840, 2011-2013, mean age: 72 years). *Hypothesis: Higher age, Black race, lower education, presence of an APOE-e4 allele, poor kidney function (eGFR<60), higher BMI, ever smoking, low physical activity, and prevalent hypertension, diabetes, CHD and stroke will be associated cross-sectionally with lower levels of Aβ42/Aβ40 ratio and higher levels of NfL, GFAP, and p-tau 181 in mid- and late-life.*

Aim 1b (longitudinal). Examine midlife demographic, behavioral, and biologic predictors of changes in plasma p-tau 181, Aβ42/Aβ40 ratio, NfL and GFAP from mid- to late-life. *Hypothesis: Higher age, Black race, lower education, presence of an APOE-e4 allele, poor kidney function (eGFR<60), higher BMI, ever smoking, low physical activity, and prevalent hypertension, diabetes, CHD and stroke will be associated with faster rates of decline in Aβ42/Aβ40 ratio and faster increases in NfL, GFAP, and p-tau 181 from mid- to late-life.*

Aim 2 (Predictive Models): Quantify change in blood biomarkers of AD pathology and neurodegeneration from mid- to late-Life in relation to (a) baseline measures and change in cognitive function from visit 5 to visit 7, (b) incident dementia in late-life, (c) subclinical markers of brain morphology and cerebral vascular damages, and (d) brain amyloid.

2a Hypothesis (cognitive decline): Faster declines in Aβ42/Aβ40 ratio and faster increases in NfL, GFAP, and p-tau 181 from mid- to late-life will be associated with lower levels of cognitive function at visit 5 and faster rates of decline in global and domain-specific cognitive function from visit 5 to visit 7.

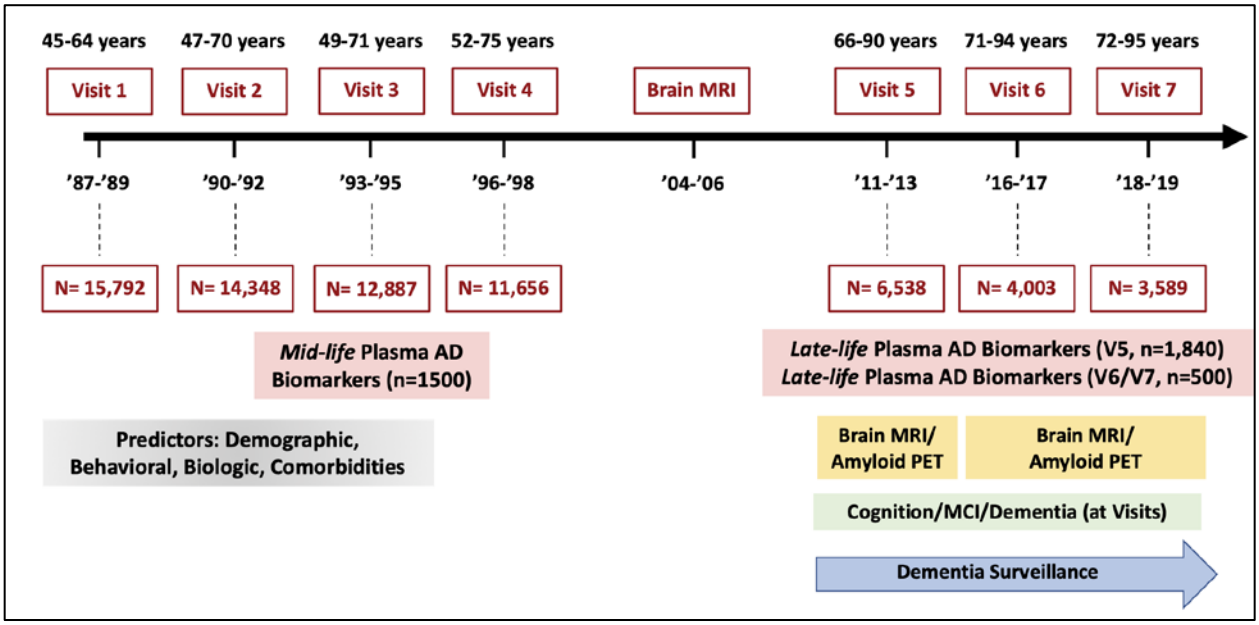
2b Hypothesis (dementia). Faster declines in Aβ42/Aβ40 ratio and faster increases in NfL, GFAP, and p-tau 181 from mid- to late-life will be associated with a higher prevalence of MCI and dementia at visit 5 and greater incidence of dementia post-visit 5.

2c Hypothesis (MRI). Faster declines in Aβ42/Aβ40 ratio and faster increases in NfL, GFAP, and p-tau 181 from mid- to late-life will be associated with greater cerebrovascular damage (e.g., larger volumes of white matter hyperintensities, greater prevalence of infarcts and microbleeds) and neurodegeneration (e.g., lower cortical thickness, lower total brain volume).

2d Hypothesis (Amyloid burden). Faster declines in Aβ42/Aβ40 ratio and faster increases in NfL, GFAP, and p-tau 181 from mid- to late-life will be associated with greater amyloid burden.

6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).

Study design (See Figure below): Prospective cohort design of blood-biomarkers in mid- to late-life and cognitive outcomes in late-life.



Exclusions: No AD blood biomarker measurements at visit 3, 5, 6, or 7

Aim 1 Objective: To document how covariates at baseline (Visit 3) are associated with the trajectory of each biomarker and evaluate whether the associations align with known risk and protective factors for cognitive decline and incident dementia.

Independent Variables. Demographic (age, sex, race, APOE-e4, education), behavioral (smoking, physical activity, BMI), biologic (cholesterol, eGFR), and comorbidity (hypertension, diabetes, CHD, stroke) predictors will primarily be measured at visit 3 (the baseline measurement of AD blood biomarkers).

Dependent Variable. Change in AD blood biomarkers: Non-normality of biomarkers will first be assessed and non-normally distributed biomarkers will be log-transformed. Linear mixed effects models will be used to estimate biomarker trajectories across the lifespan. We will fit unadjusted and covariate-adjusted linear mixed effects models using time from Visit 3 as the time scale (or age depending on model fit). Time splines will also be considered. We will examine each predictor described above plus their interaction with time on the baseline difference and change in blood biomarker levels. Each of the AD blood biomarkers will be analyzed separately at first. We will then consider models which combine multiple biomarkers as a relevant multivariate exposure.

Aim 2 Objective: To document if midlife measurements of the AD blood biomarkers and changes in the AD blood biomarkers from midlife to late-life predict late-life cognitive decline, incident dementia, measures of cerebrovascular disease, neurodegeneration, and amyloid burden.

Independent Variable. Progression in AD blood biomarkers will be examined as the independent variable in Aim 2, following the estimation of trajectories of AD blood biomarkers outlined in Aim 1.

Dependent variables.
(Aim 2a) Late-life Change in Cognitive Function from Visit 5
Cognitive function was assessed at the ARIC-NCS/visit 5 (2011-2013), 6 (2016-2017), and 7 (2018-2019) with a comprehensive neuropsychological battery. The following domains and cognitive tests were examined: memory [delayed word recall, logical memory, and incidental learning], executive functioning/processing speed [Trail Making Tests, Parts A and B; Digit Symbol Substitution Test], and language [semantic and phonemic fluency, Boston Naming Test]. Linear mixed models will be used to generate subject-specific point estimates and 95% confidence intervals (95% CI) of annualized cognitive change. Time will be defined continuously as the number of years since the baseline neuropsychological evaluation (Visit 5, 2011-2013). Linear mixed models will utilize maximum likelihood estimation with an unstructured variance-covariance matrix, and incorporate a random intercept and a random time slope. We will look at global cognition factor scores as well as domain-specific factor scores hypothesizing that AD associated markers will be more closely associated with memory deficit.

(Aim 2b) Prevalent MCI/dementia at Visit 5 and Incident dementia Post-Visit 5
Prevalent cognitive status (cognitively normal, mild cognitive impairment, or dementia) at visit 5 was determined by an expert panel among all participants who attended visit 5 and completed the neuropsychological assessments. Multivariable logistic regression models will estimate the odds of MCI or dementia at visit 5. Incident dementia following visit 5 will be based on either an adjudicated dementia diagnosis at an ARIC visit (as described) or through informant interviews or hospital discharge codes and diagnostic codes from death certificates. Cox proportional hazards regression models will be used to estimate the prospective association between AD

blood biomarkers and the onset of dementia. A sensitivity analysis will include stabilized inverse probability of censoring weights (IPCW) into the Cox model to account for informative censoring caused by competing events such as death.

(Aim 2c) Cerebral characteristics from structural brain MRI

Brain MRIs were obtained from a 3T MRI scan at visit 5/ARIC-NCS (2011-2013). Subclinical brain MRI markers of interest include white matter brain atrophy, hyperintensities, cerebral lobar and subcortical microbleeds, lacunar infarcts, silent subcortical infarcts, total brain volume, white matter hyperintensity volumes, gray and white matter volumes, and cortical thickness and volumes of brain ROIs (with special focus on the temporal- parietal lobe meta ROI). Additional outcomes include those assessed using diffusion tensor imaging to estimate white matter microstructural integrity in each ROI and overall: Fractional anisotropy (FA): lower values indicating worse white matter microstructural integrity; and Mean diffusivity (MD): higher values indicating worse white matter microstructural integrity. Multivariable logistic regression will be used to estimate the associations AD blood biomarkers with the odds of cortical or lacunar infarcts and subcortical microhemorrhages at visits 5, 6, or 7. Multivariable linear regression models will be used to estimate the mean differences in FA, MD, and log-transformed volumes of WMH, and region of interest volumes and cortical thickness between visits 5 and 6/7.

(Aim 2d) Brain Amyloid Burden from PET

Florbetapir PET scans were performed within 1 year of the brain MRI scan with magnetization-prepared rapid gradient echo (MPRAGE) used for co-registration of the PET images. Isotopes were injected 50-70 minutes before a 20-minate uptake scan. Each image was reviewed for incidental findings, image quality, and quantified for standardized uptake value ratios (SUVRs). SUVRs were obtained for each of the 34 total regions of interest (ROIs), but the primary analysis will use the global cortical measure of beta amyloid, calculated as the weighted average of the following regions: orbitofrontal, prefrontal, and superior frontal cortices, lateral temporal, parietal, and occipital lobes, precuneus, and anterior and posterior cingulate. The primary outcome is an SUVR value dichotomized at the sample median of SUVR >1.2 to indicate elevated brain amyloid burden. The value of 1.2 was chosen due to the highly skewed distribution of the data and is in line with prior ARIC-PET studies. Multivariable logistic regression will be used to estimate the associations of AD blood biomarkers with elevated amyloid burden in late-life (SUVR>1.2). Multivariable linear regression will be used to estimate the associations of AD blood biomarkers with continuous global amyloid SUVR in late-life.

Unadjusted and covariate-adjusted models will be considered in Aim 2:

- Model 1: Unadjusted
- Model 2: Adjusted for age, sex, race-center, and education as time-invariant covariates
- Model 3: Model 2 + APOE-e4 as time-invariant covariate
- Model 4 [Primary Model]: Model 3 + eGFR and BMI as time-invariant covariates (i.e. baseline value)
- Model 5: Model 4 + physical activity (MET per week) and cigarette use (current, former, never) as time-invariant covariates
- Model 6: Model 5 + diabetes, total cholesterol, HDL cholesterol, hypertension, stroke and CHD as time-invariant covariates

Methodological limitations: Attrition is of concern with close to 40% of ARIC cohort participants who were present for the visit 5 examination not examined at visits 6 and 7. Per recommendations from the NCS analysis working group, missing cognitive test scores will be imputed using multiple imputation by chained equations (MICE) models. The primary analysis will be for all-cause dementia since dementia subtype differentiation is uncertain, particularly in ARIC. However, we will also conduct analyses focused on dementia that is more likely to be due to AD.

7.a. Will the data be used for non-CVD analysis in this manuscript? ____ Yes _X___ No

b. If Yes, is the author aware that the file ICTDER03 must be used to exclude persons with a value RES_OTH = "CVD Research" for non-DNA analysis, and for DNA analysis RES_DNA = "CVD Research" would be used? ____ Yes ____ No
(This file ICTDER03 has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript? __X__ Yes __ __ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER03 must be used to exclude those with value RES_DNA = "No use/storage DNA"? _X___ Yes ____ No

8.c. If yes, is the author aware that the participants with RES_DNA = 'not for profit' restriction must be excluded if the data are used by a for profit group?
__X__Yes ____No

9.The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at:
http://www.cscc.unc.edu/ARIC/search.php

___X___ Yes _____ No

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

MP#3983 (Sullivan): Late-life plasma biomarkers for Alzheimer's disease and related dementias in association with neurocognitive and MRI outcomes

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? __X__ Yes ____ No

11.b. If yes, is the proposal
__X_ A. primarily the result of an ancillary study (list number* #2020.27)

___ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* _____ _____ _____)

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

12a. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire. Agreed

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is your responsibility to upload manuscripts to PUBMED Central whenever the journal does not and be in compliance with this policy. Four files about the public access policy from http://publicaccess.nih.gov/ are posted in http://www.cscc.unc.edu/aric/index.php, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to Pubmed central.

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