Manuscript Proposal #4101

PC Reviewed: 8/9/22	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Proteomic aging clock and brain structure, cognitive decline and the risk of dementia: Atherosclerosis Risk in Community Study

The manuscript is based on the approved ancillary proposal 2021.24 and the pending R21 grant entitled "Proteomic aging clock and brain structure, cognitive decline and the risk of Alzheimer's Disease and related dementias."

b. Abbreviated Title (Length 26 characters): Proteomic age acceleration and dementia risk

2. Writing Group:

Writing group members: Anna Prizment, Sanaz Sedaghat, Shuo Wang, Weihua Guan, Weihong Tang, Pam Lutsey, Behnam Sabayan (University of Minnesota), Timothy Hughes (Wake Forest School of Medicine), Keenan Walker (National Institute on Aging), other ARIC researchers are welcome to participate.

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

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3. Timeline: We expect the first manuscript to be submitted in a year.

4. Rationale:

Currently, over 6.2 million Americans are living with Alzheimer's Disease (AD) and AD-related-dementias (ADRD), and this number is estimated to increase to 13.8 million by 2060,[1] as our population continues to age and live longer. Newly emerging evidence has demonstrated that there is considerable inter-individual variation in aging processes. Identifying blood-based aging markers that are associated with AD/ADRD risk and preclinical AD/ADRD-related phenotypes, such as cognitive decline and early brain structural abnormalities visible in brain imaging, will help to identify high-risk individuals before the onset of symptoms. This will offer opportunities for anti-aging lifestyle and treatment interventions to delay disease progression.
Aging processes can be quantified by composite metrics called aging clocks using epigenetic, proteomic, and other biomarkers.[2,3] Proteomic aging clocks (PACs)[4,5] are particularly promising aging biomarkers because proteins change with age, have a clear biological function and may be targeted by drugs. In addition, PACs are easily measured in blood and are correlated with chronological age, which makes them relevant for studying age-related disorders[4,6-8] including AD/ADRD.[7,9] Cross-sectional analyses suggest that PACs are higher in those with cognitive decline;[7,9] however, no prospective studies investigated the association of PACs and their change over time in relation to change in cognitive function, structural changes in the brain and the risk of AD/ADRD. Creating PACs before the onset of AD/ADRD is both feasible and of clinical and public health significance, because AD/ADRD has a long natural history and changes in blood proteins happen years before clinical manifestations of the disease. Thus, pre-AD/ADRD PACs may help clarify biological pathways and enable risk stratification.

Our ***main objectives*** are to create PACs and examine whether they are associated with AD/ADRD risk, cognitive decline and structural abnormalities visible in brain imaging in the Atherosclerosis Risk in Community (ARIC) cohort of White (75%) and Black (25%) adult men. As development of PACs is novel and no consensus about the best PAC has been established yet, we will examine several PACs in ARIC participants who are AD/ADRD-free. Our approach leverages the resources of the ARIC cohort with >5000 plasma proteins measured 3 times over 20+ yrs (age range 46-90 years) using the highly sensitive SomaScan assay (n=12,769 in midlife and n=5193 in late life) and well-validated information on AD/ADRD status, cognitive function and brain imaging.[10,11]

5. Main Hypothesis/Study Questions:

Our central hypothesis is that higher PAC value and PAC's change between midlife and late-life are associated with higher risk of AD/ADRD, steeper decline in cognitive function, and higher prevalence of structural abnormalities in the brain. Importantly, the associations will be examined in women and men, as prior studies suggested a heterogeneity by sex and this is a high-priority NIH research question in AD/ADRD.
We will test our hypothesis in the following specific aims:
SA1: Create PACs and assess their performance in AD/ADRD-free participants in midlife (46-70y) and late-life (66-90y). The performance of PAC will be acceptable and PAC will be considered valid if correlation between PAC and chronological age is >0.7 in AD/ADRD-free persons.
SA1a. Compute and test two published PACs[4,5] in AD/ADRD-free persons
SA1b. Create two new PACs using penalized regression model in a random set of AD/ADRD-free persons: i) first-generation PACs based on protein levels in mixed-age individuals (cross-

sectional PACs); ii) new-generation PACs based on the intra-individual change in protein levels between midlife and late-life;
The new PACs will be tested in the remaining set of AD/ADRD free participants in ARIC.

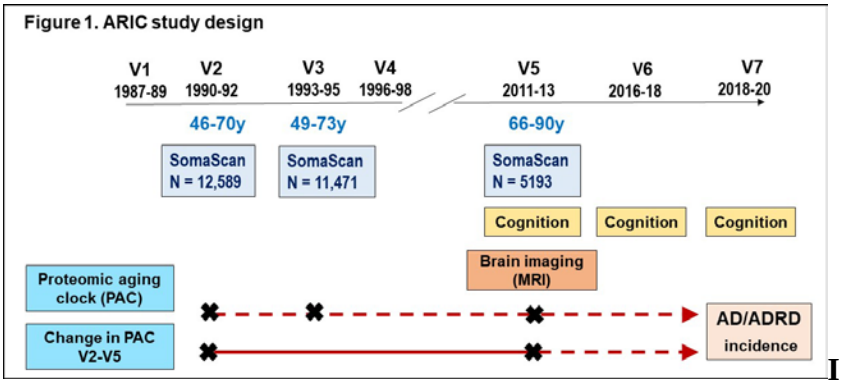
SA2: Determine prospectively whether PACs (from SA1, independent of chronological age) and PACs' change from midlife to late-life are associated with the risk of AD/ADRD both overall, and by sex and race, in participants who do not have AD/ADRD at baseline. In an exploratory analysis, we will also examine all the associations with different AD/ADRD types (AD, vascular dementia, and other subtypes of dementia)[12] to elucidate potential pathophysiology.

SA3: Determine whether PACs (from SA1, independent of chronological age) and PACs' change from midlife to late-life are associated with decline in cognitive function and with prevalence of structural changes visible in brain imaging (such as parenchymal loss, white matter hyperintensities, and small infarcts) in late-life, overall, by sex and race in AD/ADRD-free participants.

We expect that longitudinal PACs based on the change from midlife to late-life will be more strongly associated with AD/ADRD, cognitive decline and higher prevalence of structural abnormalities in the brain compared to cross-sectional PACs.

Impact: Understanding the relations between aging and AD/ADRD is essential for risk stratification and personalized prevention and treatment via lifestyle changes and anti-aging therapies.[13]

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).



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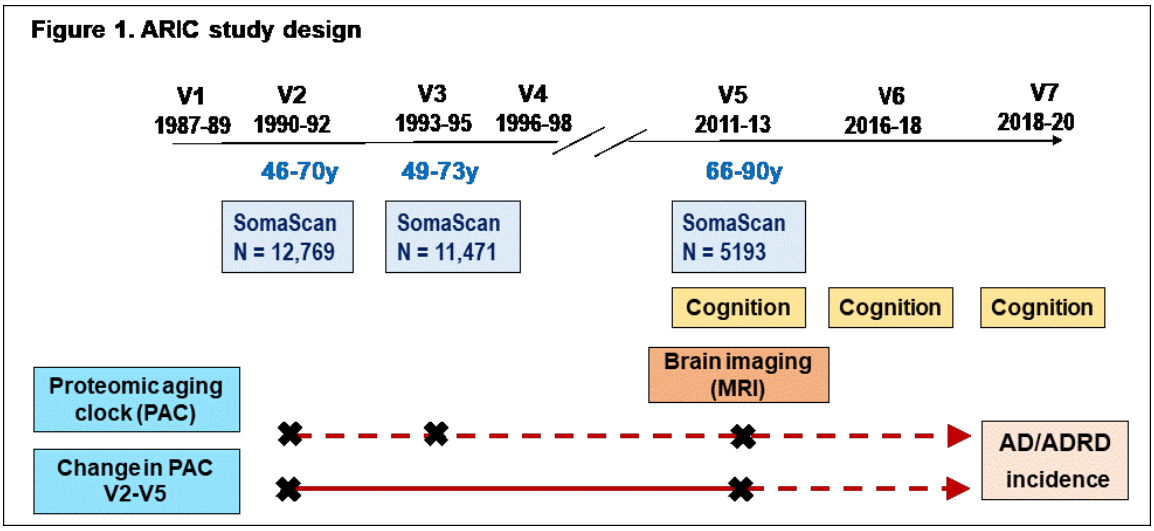
Inclusion criteria: The analytic sample will include participants who were free of AD/ADRD at baseline (Visit 2), and who have the measurement of proteins at Visit 2 using the SomaScan assay.

Exposure: Circulating levels of proteins will be extracted from the SomaScan assay (v.4) (SomaLogic company).[11,14] Using this assay, the ARIC study has recently measured more than 5000 plasma proteins in frozen plasma samples collected at Visit 2 (1990-1992, N= 12,589),

Visit 3 (1993-1995, N=11,340) and Visit 5 (2011-2013, N=6538) (Median CV range across ~5 proteins measured from ARIC visit 3 samples: 6.9 – 8.3%). The proteins constituting the proteomic aging clock will be assessed at baseline (Visit 2) and updated at (Visit 3 and Visit 5, where appropriate). In all the analysis, we will use the log transformed values of proteins (Figure 1).



Outcomes:

AD/ADRD. AD/ADRD was assessed using well-validated, standardized battery of cognitive measures supplemented by AD/ADRD surveillance in between visits, and hospital discharge or death certificate previously described in detail.[12,15-18] For persons examined at ARIC visits, AD/ADRD diagnosis is consistent with the National Alzheimer's Coordinating Center (NACC) procedures and the criteria are quantitative, requiring for AD/ADRD: **(i)** cognitive domain test failure (≥2 domains scoring ≥1.5 SD below ARIC-community specific norms for age, race and education level), **(ii)** 10-test cognitive score decline (≥0.055 SD/year), and **(iii)** functional disability (FAQ>5 or CDR Sum of Boxes>3), with expert committee review and adjudication for disagreements. ***For persons who missed a visit***, diagnosis was made via the Telephone Interview for Cognitive Status (TICS) or telephone-based CDR-based informant interview using thorough "hot pursuit" (every-6-month) surveillance. ***For those not evaluated in person and unable to undergo a telephone interview***, an informant interview was completed especially if there was suspicion of cognitive impairment or insufficient data. Together, these tests were shown to capture most common AD/ADRD and the types including AD, mixed dementia, vascular dementia and Lewy Body dementia.[12] In addition, for all participants diagnosis was made using hospital or death certificate ICD codes. The information on AD/ADRD was reviewed by a physician (neurologist or geriatrician) and a neuropsychologist.[12] The clinician reviewers agreed with the algorithmic diagnosis 94% of the time.[12] The ***AD/ADRD onset*** was the earliest date determined by AD/ADRD surveillance, or by hospital discharge or death certificate codes.

Cognitive function*:* We will use information on different cognitive function domains at V5, V6, and V7 (earlier ARIC visits used different cognitive tests) measured in all participants. A neuropsychological test battery was administered to participants by trained examiners in a standardized order during one session in a quiet room. Tests administered to evaluate each of

multiple cognitive domains are presented in **Table 1**. We will use factor analysis to derive scores for memory, executive function, language, visuospatial domains and overall cognition (combination of all cognitive domains).[19,20]

Brain imaging (Table 1)*:* MRI has been performed on 3T Siemens systems at Visit 5. The following sequences were obtained from all participants: MP-RAGE, Axial T2*GRE, Axial T2 and FLAIR (fluid-attenuated inversion recovery). Brain volumes were measured on MP-RAGE sequences using FreeSurfer,[21] image analysis software. Cerebral infarctions and microbleeds were identified and measured by a trained imaging technician and confirmed by radiologists.[22] Cortical infarctions were defined using FLAIR sequences as a hyperintense lesion ≥5 mm in size in the greatest dimension in cortical grey matter, which may extend to underlying white matter. Lacunar infarctions were defined as a hyperintense subcortical lesion with a dark center (≥3mm and ≤15mm in size) within the white matter, infratentorial, or central grey/capsular regions that is distinguishable from perivascular space. Cerebral microbleeds were defined using a T2* GRE MRI sequence. White matter hyperintensity volume was derived from proton density-weighted images using a quantitative computer-aided segmentation program which uses an algorithm to segment FLAIR images (FLAIR-histoseg) and measure the volumetric burden of leukoaraiosis. Brain imaging data underwent a thorough and extensive data flow and Quality Control which is described in detail previously.[15,22,23]

Table 1. Brain imaging markers in MRI, cognitive function and covariates in ARIC

Brain MRI	Regions
Structure, vascular/ small vessel disease	Total, white and gray matter volume White matter hyperintensity Cerebral microbleeds (deep cerebral, infratentorial) Infarcts (cortical infarcts, subcortical infarcts and lacunar infarcts)
Cognitive Domain	**Tests**
Screener	Folstein Mini-Mental State Examination (MMSE)
Memory	Delayed word recall, Logical Memory I and II, Wechsler Memory Scale revised Incidental learning
Executive function/ Speed of processing	Trails A, Trails B, Digit Span Backward, Digit Symbol Substitution Test, Word Fluency (FAS)
Language	Category Fluency (animals),Boston Naming (15 item)
Visuospatial	Clock Drawing, CERAD Constructional Praxis
Overall cognition	Combination of above domains derived from factor analysis
Characteristics	**Covariates**
Demographic	Age, sex, race, study center
Anthropometric	Body mass index (BMI)
Lifestyle	Smoking history, alcohol intake, physical activity, education
Clinical	Use of medications such as aspirin, metformin, and hormone therapy for women, medical history, kidney function using glomerular filtration rate

Other covariates of interest: Demographic, anthropometric and lifestyle characteristics of interest such as chronological age, sex, race, education, BMI, collected at baseline, smoking status, cigarette years of smoking, alcohol use/intake, physical activity, and diabetes, will also be extracted at Visit 2 and updated as needed during follow-up Information about estimated glomerular function and APOE status will be also extracted

Statistical analysis

Overview: We will stratify all analyses by sex, race/ethnicity and APOE status (1 or 2 ε4 alleles versus no ε4 alleles), with test for interaction with PAC on a multiplicative scale. We will use inverse probability attrition weighting (IPAW) to limit selection bias attributed to participation in brain imaging study, those who died or did not participate in the follow-up visits.

Specific aim 1: Create PACs and assess their performance in AD/ADRD-free participants in midlife (46-70y) and late-life (66-90y).

SA1a. Assessing two published PACs. Using proteins and regression coefficients from the two published PACs,[4,7] we will compute these PACs in ARIC by summing up protein levels multiplied by

regression coefficients as weights: $\boldsymbol{Proteomic\ aging\ clock} = \boldsymbol{\beta_0} + \sum_{k=1}^{n} \boldsymbol{\beta_k Protein_k}$ **(Eq. 1)**. To examine PAC's validity, we will use standard approach used in the studies of aging clocks, i.e. test their correlation with chronological age and the association with total mortality.

SA1b. The creation of new PACs will include the PAC's construction in ***the training set*** of 2000 (based on previous studies of similar size)[4] of randomly selected AD/ADRD-free ARIC participants and ***testing*** PAC's performance in remaining AD/ADRD-free ARIC participants. We will construct two new PACs (**Table 2):**

Table 2. Construction of two new PACs in the proposed study
i) first generation cross-sectional PACs will be created at each Visit (V2, V3, and V5) using penalized regression (elastic net, using R package 'glmnet') in 2000 randomly selected ARIC participants who ere AD/ADRD-free until the end of follow-up. The model will be trained against chronological age (outcome) and all SOMAscan proteins levels as predictors.[72,73] The elastic net model will perform protein selection and create a parsimonious model.[5,24,74]
ii. new generation PACs will be created based on the intra-individual change in the level of each of ~5000 proteins between V2 and V5 (over 20+ years of follow-up) in 2000 randomly selected AD/ADRD-free ARIC participants.[20,53] To maximize the duration of follow-up, this PAC will be constructed using proteins measured at V2 (and not V3) and V5 in three steps: (1) calculate intra-individual changes in blood levels, (2) compute a standardized ratio by dividing the mean difference for each protein by its SD, and (3) rank the protein based on their standardized ratios. Ours and previous studies[4,41] suggested that ~900-1000 proteins are associated with age. To avoid missing any important proteins, we plan to select top 1250 proteins with the highest standardized ratios to be included into elastic net regression model as predictors, and chronological age will be the outcome.

Examining PACs' performance. For all remaining AD/ADRD-free ARIC participants, we will compute a weighted PAC by multiplying protein levels by the coefficients obtained in the training set (Eq. 1). We will estimate the performance of each PAC using ***two main criteria for a PAC*** to serve as a good biomarker of biologic aging (i.e. be valid): ***(1)*** be correlated with chronological age in the testing dataset with $r \geq 0.7$)[24,25,26] and ***(2)*** be associated with total mortality independent of chronological age.[27]

Age acceleration will be created to study associations with the outcomes independently of age, by regressing PAC on chronological age (residuals) in all participants. A ***positive value of age acceleration*** indicates that the proteomic age is higher than the person's chronological age.[28] This is a standard, well-validated approach used in the studies of aging clocks (EACs[24,25] and PACs[4,5,7]).

Specific aim 2. Determine prospectively if PACs (independent of chronological age, i.e. age acceleration) **and PACs' change from V2 to V5 are associated with AD/ADRD risk,** overall, by sex and race.

Association between age acceleration and AD/ADRD risk (N=2760 cases). Cox proportional hazards regression will be used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) to study age acceleration associated with risk of AD/ADRD in the whole study after excluding the training set. In the main analysis, the exposure will be age acceleration at V2. In additional analyses, age acceleration will be treated as a time-dependent variable accounting for values at V3 and V5. The acceleration is a continuous variable, but will be transformed or categorized into quartiles if there is a nonlinear relationship with AD/ADRD risk. The follow-up will be from V2 until the diagnosis of AD/ADRD, loss to follow-up, death, or the end of follow-up on December 2019, whichever occurred first. The proportional hazard assumption will be tested by Schoenfeld residuals. The analysis will account for competing risk of death using cause-specific proportional hazard analysis or Fine-Gray methods.[29] Two models will be used to study age acceleration and risk of AD/ADRD: **Model 1**: adjusted for sex, race, and study center; **Model 2:** further adjusted for education/income, BMI, smoking status, alcohol intake, physical activity and kidney function. In an exploratory analysis, we will compare associations of age acceleration with different types of

AD/ADRD (specifically AD, vascular dementia, and mixed dementia available at V5) to clarify the pathophysiology of the association.

Association between the change in PAC from midlife (V2) to late-life (V5) and AD/ADRD risk (N=707 cases)**.** We will repeat all the analyses using the pre-diagnostic PAC's change as an exposure. PAC's change will be created as the intra-individual change in PACs values between the visits. The follow-up will be from V5 until the diagnosis of AD/ADRD, loss to follow-up, death, or the end of follow-up on December 2019, whichever occurred first. Of note, ***there is a difference between two concepts, PAC's change between visits and age acceleration.*** PAC's change is longitudinal, while age acceleration shows deviation of estimated age (PAC) from chronological age age.[4,5,7,24,25]

Power calculation for SA2a and SA2b are presented in Table 3.

Table 3. Power for SA2. Minimum detectable AD/ADRD associated with 1SD of PAC acceleration risk (80% power, 2-sided α= 0.05).

Outcome	No. of persons[a]	No. of cases	Minimum risk	Minimum risk for interaction with sex[b]
SA2a: ADRD/AD (from V2--2019)	N~7260	2760	1.07	1.18
SA2b: ADRD/AD (from V5--2019)	N-2800	707	1.13	1.38

[a]After excluding the training set of 2000 AD/ADRD-free persons at the corresponding visit; [b]The effect size is the odds ratio of the interaction assuming OR=1.3 for the PAC main effect term and OR=1.2 for AD/ADRD associated with sex

Specific aim 3. Determine whether PACs (independent of chronological age, i.e., age acceleration) and PACs' change from midlife to late-life are associated with decline in cognitive function and with structural abnormalities visible in brain imaging in AD/ADRD-free participants.

a) Association of age acceleration with cognitive function (V5) and annual decline in cognitive function from V5 to V6 and V7 among 3063 AD/ADRD-free participants at V5. Analyses will be done both cross-sectionally (cognitive function at V5) and longitudinally (annual decline in cognitive function over V5, V6, and V7). We will analyze different cognitive domains and overall cognitive function, separately **(Table 2).** We will use linear regression models to study cross-sectional association of age acceleration and PAC's change with cognitive function at V5. For change in cognitive function over time, linear mixed effects model (with an unstructured covariance matrix) will be used to estimate the annualized rate of decline from V5 to V6 and V7 in each cognitive domain and overall cognitive function.

b) Association of age acceleration with structural changes visible in brain imaging in a cross-sectional analysis among 1601 AD/ADRD-free participants at V5. We will estimate the association of age acceleration with white matter and gray matter volumes (continuous, linear regression), white matter hyperintensities (continuous, linear regression models), cerebral microbleeds (yes/no; n cases=387), subcortical (yes/no; n cases=306) and cortical (yes/no; n cases=172) and lacunar infarcts (yes/no; n cases=289) (all will be analyzed by logistic regression models). We will additionally use cerebral small vessel disease composite score described previously[30] (ordinal, via ordered logistic regression) which reflect the cumulative presence of elevated white matter hyperintensity, cerebral microbleeds, and lacunar infarcts, i.e. the overall burden of cerebral small vessel disease as outcome. White matter hyper-intensity volume will be log-transformed to adjust for skewness. Models with white and gray matter volume and white matter hyperintensity as outcomes, will be additionally adjusted for total intracranial volume in addition to the covariates discussed in SA2 to control for participants head size.

Power calculation for SA3. For global cognitive function at V5 and for change in cognitive function from V5 to V7 (both: continuous variables), among 3063 AD/ADRD participants (at V5, after excluding the training set), there will be 80% power (two-sided alpha=0.05), to detect the effect size of 0.003, where effect size is the proportion of variance in the outcome explained by age acceleration. For structural changes at V5, among 1601 participants, there will be 80% power (two-sided alpha=0.05) to detect the effect size of 0.005.

Pathway analysis. While our main objective is to examine how PAC predict AD/ADRD risk, identifying specific age-related proteins will elucidate the mechanisms explaining the role of aging in the development of AD/ADRD and may provide insights for actionable targets for anti-aging therapies.[31] Thus, first, we will test whether each of the top age-related proteins in each PAC is associated with the outcomes after adjusting for the other top proteins. Then, we will explore biological processes, molecular functions, and cellular components relevant to specific proteins significantly associated with PACs in our study by conducting Ingenuity Pathway Analysis (IPA, QIAGEN Inc).[32,33] This analysis will be used to cluster age-related proteins into pathways and functional groups. The Fisher right-tailed exact test will be used to test for enrichment of the identified proteins in specific pathways, compared to the pathway explained by chance alone. A cutoff $\log_2$ ratio of >1.0 and <−1.0 (2-fold change in abundance) and a *P* value cutoff of 0.05 will be used after applying Benjamini-Hochberg FDR adjustment for multiple comparisons. We will also use pathway analyses to identify upstream regulators, i.e., genes, transcription factors, proteins that are known to regulate the abundance of large group of PAC proteins. These upstream regulators have an increased likelihood of being mechanistically relevant to accelerated PAC.

7.a. Will the data be used for non-ARIC analysis or by a for-profit organization in this manuscript? ____ Yes _X__ No

b. If Yes, is the author aware that the current derived consent file ICTDER05 must be used to exclude persons with a value RES_OTH and/or RES_DNA = "ARIC only" and/or "Not for Profit" ? ____ Yes ____ No
(The file ICTDER 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? ____ Yes __X__ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the current derived consent file ICTDER05 must be used to exclude those with value RES_DNA = "No use/storage DNA"? ____ 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/mantrack/maintain/search/dtSearch.html

__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)?

MP3057 Repeatability and Longitudinal Variability of the Plasma Proteome.
MP3327 A proteomic analysis of incident dementia: The ARIC Study
MP3739 Proteomic age acceleration and cancer incidence: The Atherosclerosis Risk in Communities Study
MP3848 Proteomic age acceleration and mortality in cancer survivors: The Atherosclerosis Risk in Communities Study

MP4081 Proteomic age acceleration and dementia and frailty in cancer survivors: The Atherosclerosis Risk in Communities Study

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* _
AS2017.27 Proteomic longitudinal ARIC study: SOMAscan of multiple visits
AS2021.24 Proteomic aging clock and dementia: Atherosclerosis Risk in Community Study.

_ __ 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 https://sites.cscc.unc.edu/aric/approved-ancillary-studies

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.

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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