ARIC Manuscript Proposal #3610 (revised)

PC Reviewed: 9/8/20 **Status: _____** **Priority: 2**
SC Reviewed: _____ **Status: _____** **Priority: ____**

1.a. Full Title: Quantitative changes in the plasma proteome during the menopausal transition: The Atherosclerosis Risk in Communities (ARIC) study

b. Abbreviated Title (Length 26 characters): Menopause and proteomics

This is one of 3 proposals which are the topic of an ARIC diversity supplement awarded to Dr. Appiah

2. Writing Group:
Writing group members: Duke Appiah, Pamela Schreiner, James Pankow, Guy Brock, Weihong Tang, Faye Norby, Erin Michos, Christie Ballantyne and Aaron Folsom

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

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3. Timeline: We aim to complete the manuscript a year from the time of approval.

4. Rationale:

Globally, the incidence and mortality of cardiovascular diseases (CVD) tends to be higher in men than women (1). However, the relative risk for obesity and hypertension morbidity and mortality, which are major risk factors for CVD, is higher for women than men in the US (2). Despite the recent progress made in identifying and narrowing the gaps in cardiovascular outcomes between men and women, understanding of underlying factors and mechanisms that explain differences in incidence and presentation of CVD between the sexes are limited (3).

Some studies suggest that menopause plays important roles in the sex disparities reported for CVD outcomes (4). The prevalence of CVD especially coronary heart disease (CHD) is greater in men than in women until menopause, when the prevalence of CVD increases in women until it exceeds that of men (5). Because of the observation of CVD risk coinciding with menopause, it has been suggested that endogenous hormones such as estrogen are cardio-protective (5, 6). However, recent evidence suggests that these relationships are more complex than thought, as several other factors beyond endogenous hormones such as metabolic changes also occur during and after the time of menopause. Gaining a better understanding of the mechanisms through which menopause is associated with CVD independent of chronological aging holds promise to understanding the sex disparities in CVD events between men and women.

Genome-wide association studies among women of different races and ethnicities have observed a strong genetic component in the timing of menopause with more than 50% of the variation in menopause onset attributed to genetic factors (7). Some previous studies with small samples and using micro single radial immunodiffusion methods have observed differences in proteins between premenopausal and postmenopausal women (8) However, no large epidemiologic studies have been conducted that comprehensively profiles the plasma proteome during the menopausal transition to uncover changes in protein biomarkers that relate to menopause independent of chronological age.

Therefore, the objective of this proposal is to characterize quantitative changes in the plasma proteome across the menopausal transition in women enrolled in the Atherosclerosis Risk in Communities (ARIC) study. The ARIC cohort has a large number of women who transitioned from pre-menopause to post-menopause based on self-report during follow-up and performed a comprehensive quantitative proteomic profiling of the plasma proteome in these women. This offers the opportunity to evaluate changes in protein level during the menopausal transition.

5. Main Hypothesis/Study Questions:

1) **What plasma proteins change significantly during the menopausal transition?** We hypothesize that the levels of some novel proteins will change significantly more among women who transition naturally from premenopausal status to postmenopausal status from ARIC visit 2 to 3, compared to women who remain premenopausal at these two time points.

2) **Do the changes of plasma protein levels during the menopausal transition differ by race?**

We hypothesize that there will be significant differences in proteins that changed significantly from ARIC visit 2 to 3 between black and white naturally menopausal women. This is due to the observation that the age at onset of menopause varies by race.

3) **Do the changes of plasma protein levels during the menopausal transition differ by smoking status?**
   We hypothesize that there will be significant differences in proteins that changed significantly from ARIC visit 2 to 3 between current, former and never smokers at visit 2 since smoking has been reported to accelerate the onset of menopause.

4) **Does hormone therapy use influence changes in plasma protein in postmenopausal women?**
   We hypothesize that among women who were naturally postmenopausal at ARIC visit 2, there will be larger changes in novel proteins from ARIC visit 2 to 3 for women wo were currently using hormone therapy than former or never users at visit 2.

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: Longitudinal study using data from ARIC visits 2 and 3.

Exclusions: The analysis will be restricted to women who attended both ARIC visits 2 and 3. About 7944 women attended ARIC visit 2 and 4331 of them reported reaching menopause naturally. There were 7044 women who attended both ARIC visits 2 and 3. Among these women, 1464 were premenopausal at ARIC visits 2 with 443 reaching natural menopause by ARIC visit 3. Women who had surgical menopause or without plasma proteomic data at these two time points as well as all non-human proteins detected on the SOMAScan due to cross-reactivity of aptamers will be excluded.

Exposure: Women will be considered to be postmenopausal if they reported not having had a menstrual period within the 2 years before the current ARIC visit.

Outcome: Protein biomarkers (~5000) assessed using SOMAscan (v. 4) multiplexed aptamer-based proteomics platform.

Covariates (Visits 2 and 3)

Demographic variables: age, race, ARIC field center, and educational level (years of education).

Anthropometric measures: waist circumference and body mass index.

Reproductive factors: age at menarche, parity, hysterectomy status, oophorectomy status, and hormone therapy use.

Health behavioral/lifestyle factors: smoking status (never, current, former) and pack years, physical activity (Baecke PA scores) and alcohol use.

Health history and conditions: systolic blood pressure and anti-hypertensive medication use, lipid-lowering medication use, anticoagulants, diabetes, medication for diabetes and cancer.

Blood biomarkers: total and HDL cholesterol, fasting glucose and kidney function using estimated glomerular filtration rate (eGFR).

Statistical Analysis: Standard descriptive statistics will be used to describe the continuous and categorical variables for participants in the analytic sample. We will use graphical techniques to explore the distribution of proteins and change between visits 2 and 3. Since ARIC uses log base 2 transformations for all proteins on the SOMAscan, we will apply this to all of our initial analyses. For proteins that change significantly during the menopausal transition, we will explore other transformations for non-normal distributions. . To test the hypotheses for Study questions 1, 2, 3 and 4, ordinary least squares regression techniques will be employed to test for differences between groups (i.e. those who became postmenopausal versus those who stayed premenopausal). The outcome for these analyses will be change in plasma proteins from ARIC visits 2 to 3. To test for effect modification of race (Study question 2) or smoking status (Study question 3) with menopause on changes in proteins, interaction terms between race (black or white) or smoking status at visit 2 (never, former, current) and postmenopausal status at visit 3 (yes, no) will be included in the model. The influence of change in smoking status on change in proteins will also be evaluated. For Study question 4, the 3-year changes in novel proteins from ARIC visit 2 to 3 will be compared between hormone therapy (HT) use status according to current, former, or never use of HT at visit 2. Additional analyses will be conducted taking into account changes in HT use from ARIC visit 2 to 3; namely, changes in novel proteins will be compared among postmenopausal women in 5 groups: women who did not use HT at both visit 2 and 3 (nonusers); women who used HT at visit 2 but not at visit 3 (discontinuers, short-term); women who were former users at visit 2 and never used HT at visit 3 (discontinuers, long-term); women who began HT after visit 2 (initiators); and women who used HT at both visits (continuing users). We will also evaluate the influence of regimens of HT such as estrogen alone or estrogen plus progesterone on changes in proteins. All models will control for the influence of chronological age and eGFR with other confounders (listed above) adjusted whenever appropriate. Results from these analyses will be compared to regression models where the outcomes will be proteins measured at ARIC visit 3 with their corresponding proteins measured at visit 2 modelled as a covariate. Finally, we will evaluate changes in these proteins at visit 2 to 3 comparing women who transitioned to menopause to those who remained premenopausal at these two time points and those who remained postmenopausal at these two time points.

ARIC has measured approximately 5000 plasma proteins at the specified ARIC visits. Therefore, adjusted p values will be calculated to control the false discovery rate for the 5000 comparisons that will be made using Bonferroni correction and other methods such as Benjamini and Hochberg's method. It is possible that even after such adjustments of the p values, many significant changes in proteins that are not in obvious pathways for onset of menopause will be discovered. To help explain these pathways and to identify any possible causal or meaningful

associations between changes in proteins and menopause, Ingenuity Pathway Analysis will be performed.

Several sensitivity analyses will be performed. First, to validate menopausal status, levels follicle stimulating hormone levels on the SOMAscan panel will be compared between premenopausal and postmenopausal women. Self-reported postmenopausal status has good agreement with clinical measures of menopause. Second, analysis categorizing menopausal status into pre-, peri- and post-menopausal status at ARIC visits 2 and 3 will also be performed to evaluate changes in novel proteins across these three categories. t Thirdly, if numbers permit, all analyses will be repeated excluding women with cancer and those taking anticoagulants, antihypertensive and cholesterol-lowering medications as well as medication for diabetes at ARIC visits 2 and 3 as they can potentially influence the levels and changes in proteins. If numbers are insufficient, we will adjust for these instead. Finally, we will also perform analyses to address the potential issues of drift, variable calibration across visits and informative missing-ness that might lead to bias in this study.

Limitations: The modified aptamer technology used in ARIC provides relative quantification instead of absolute quantification. The possibility of protein degradation during long-term storage cannot be excluded. A validation studies in ARIC did not support widespread protein degradation across visits (9). Findings from this proposed research project would benefit from external validation for confirmation. Unlike most cohorts which evaluated menopausal status using used self-report of no menstrual period in the past year, ARIC defined postmenopausal status as no menstrual period within the past 2 years before the visit. This definition is conservative and ensured that premenopausal women were not classified as postmenopausal. However, we acknowledge that the precision of recollecting age at menopause appears to decrease as time since menopause increases With women enrolled between the ages of 45-64 at visit 1, the believe that issues with recollection if present will be minimal. We plan to compare the distribution of the levels of hormones on the SOMAscan panel related to menopause such as follicle stimulating hormone between premenopausal and postmenopausal women to evaluate the concordance of self-reported menopausal status with clinical definitions of menopausal status

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)?

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

11.b. If yes, is the proposal

___ A. primarily the result of an ancillary study (list number* _____)

___ 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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