

## ARIC Manuscript Proposal #3443

PC Reviewed: 8/13/19  
SC Reviewed: \_\_\_\_\_

Status: \_\_\_\_\_  
Status: \_\_\_\_\_

Priority: 2  
Priority: \_\_\_\_\_

**1. a. Full Title:** Association between *APOLI* risk alleles, troponin levels, and BNP among African Americans in The Atherosclerosis Risk in Communities Study

**b. Abbreviated Title (Length 25 characters):** *APOLI*, Troponin, and BNP

### 2. Writing Group:

Writing group members: Aditya Surapaneni, Shoshana Ballew, Josef Coresh, Christie Ballantyne, Elizabeth Selvin, Morgan Grams, **others welcome**

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]**

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### 3. Timeline:

Analysis will begin upon receipt of the data and approval of the manuscript proposal. Manuscript will be written and submitted for ARIC Publications Committee review within one year of manuscript proposal approval.

### 4. Rationale:

*APOLI* risk alleles have been associated with an increased risk of kidney disease in African Americans, with a two-fold higher risk of developing end-stage renal disease (ESRD) reported in people with 2 risk alleles compared to those with 0 or 1 risk alleles.<sup>1-5</sup> However, not everyone with the high risk genotype progresses to disease. It has been suggested that a “second hit”, an exposure that modifies the risk of *APOLI*, may be required for a decline in kidney

function.<sup>6-8</sup> Identifying such exposures that might be modifiable is therefore an important topic for investigation.

Cardiovascular damage may play a role in precipitating CKD progression among patients with *APOLI* high-risk genotypes. A recent meta-analysis showed a small but statistically significant association between *APOLI* risk alleles and cardiovascular disease in demographically adjusted, but not fully adjusted, models.<sup>10</sup> However, it did not test whether the development of cardiovascular disease might hasten eGFR decline, nor did it evaluate subclinical markers of cardiovascular disease.

Troponin-T and N-terminal pro brain natriuretic peptide (BNP) are markers of cardiac injury that have been associated with the development of ESRD.<sup>9</sup> Troponin-I is another marker of cardiac injury that has been studied in the context of ESRD.<sup>11</sup> Although *APOLI* risk alleles are expressed in the vasculature, to our knowledge, no previous studies have investigated the association between *APOLI* risk alleles and levels of these markers, or whether higher levels of troponin-T, troponin-I, or BNP portend higher risk of ESRD associated with *APOLI* risk alleles in African Americans.

We aim to examine the associations of *APOLI* risk alleles with troponin-T, troponin-I, and BNP and to examine whether they modify the risk of *APOLI* risk alleles for ESRD.

## **5. Main Hypothesis/Study Questions:**

To test whether *APOLI* risk alleles are associated with subclinical cardiac damage as assessed by levels of troponin-T, troponin-I, and BNP, and to test whether the risk of the *APOLI* high-risk genotype for ESRD varies by levels of troponin-T, troponin-I or BNP.

We hypothesize that *APOLI* risk alleles are associated with more subclinical cardiac damage, as assessed by higher levels of troponin-T, troponin-I, and BNP, and that the risk of *APOLI* high-risk genotype for ESRD is higher with higher levels of these markers.

## **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:**

We will first conduct a cross-sectional analysis of the ARIC cohort, using study visit 2 (1990 – 1992) to assess the association between *APOLI* risk alleles and markers of cardiac damage. Then, using visit 2 as baseline, we will conduct a prospective analysis for the outcome of ESRD with follow up through December 31<sup>st</sup> 2017. In sensitivity analyses, we will investigate other visit dates as baseline, particularly since troponin-I and albuminuria are only available at visit 4 and 5.

### **Study Population:**

The study population will include African Americans in the ARIC cohort at visit 2 that have available data on *APOLI* risk alleles and either BNP or troponin-T measures at visit 2. In sensitivity analyses, we will perform the same analyses for the populations at visits 4, 5, and 6 with available data. African Americans at visits 4 and 5 with available troponin-I data will constitute the study population for the *APOLI* troponin-I analysis.

### **Exposure:**

*APOLI* high risk-genotype, defined as two G1 alleles, two G2 alleles, or one G1 and one G2 allele. The alleles were genotyped using Taqman assays. The G1 haplotype was defined by two nonsynonymous missense variants (rs73885319, S342G, and rs60910145, I384M), and the G2 haplotype was defined by a 6 base pair in-frame deletion (rs71785313, N388del:Y389del).

Cardiac troponin-T from visit 2 was measured from stored serum samples using a Roche Elecys 2010 Analyzer (Roche Diagnostics) at the University of Minnesota in 2012-2013. Troponin-T from visit 4 was measured in stored supernatant samples at Baylor College of Medicine in 2010 using an electrochemiluminescent immunoassay implemented on a Roche Cobas e411 analyzer. Troponin-T from visit 5 and visit 6 was measured in serum samples on a Roche Cobas e411 analyzer using a highly sensitive assay. (Elecys Troponin T Gen 5 STAT, Roche Diagnostics, Indianapolis).

BNP from visit 2 was measured from stored serum from samples and analyzed using a sandwich immunoassay method (Roche Diagnostics) implemented on a Roche Elecys 2010 Analyzer at the University of Minnesota. BNP from visits 4, 5, and 6 was measured on the automated Cobas e411 analyzer (Roche Diagnostics) using an electrochemiluminescent immunoassay with a measurement range of 5-35,000 pg/mL and a limit of quantitation of 35 pg/mL

Troponin-I was measured in EDTA plasma samples in 2016-2016 from visit 4 and visit 5 using a chemiluminescent immunoassay (Architect Stat Troponin-I; Abbott) on an automated chemistry analyzer (Architect *i* 2000sr; Abbott).

These latest generation high sensitivity troponin assays are not the assays typically used for diagnosis of MI in clinical use.

### **Outcomes:**

The primary outcome for the prospective analysis is incident ESRD. Incident ESRD will be defined as the initiation of dialysis therapy or transplantation as identified by the United States Renal Data System (USRDS) registry. As a secondary outcome, we will examine the decline in kidney function as assessed by slopes in estimated GFR.

### **Statistical Analysis:**

Descriptive statistics will be used to examine baseline characteristics of the study participants according to *APOLI* risk status. Differences will be tested using chi square test for categorical variables and t-tests for continuous variables.

Linear regression will be used to estimate the association (beta coefficients, 95% confidence intervals) between *APOLI* risk alleles and BNP and troponin-T levels at the baseline visit 2. These associations will be evaluated again at subsequent visits 4, 5 and 6. Associations between *APOLI* risk alleles and troponin-I will be evaluated at visits 4 and 5. We will address the issue of values that could not be detected by imputing with half the lower limit of detection. We will also model the levels categorically, for instance, as tertiles.

Cox models will be used to estimate the association (Hazard Ratios, 95% confidence intervals) of the *APOLI* high risk genotype with the development of ESRD and the modification of that risk by troponin-T and BNP levels. In sensitivity analyses, we will model *APOLI* risk alleles using a genotypic model which treats the 0, 1 and 2 risk alleles as separate categories, and using an additive model which models the number of alleles linearly. We will also model the association of *APOLI* risk alleles with the development of ESRD accounting for the competing risk of death using the Fine and Gray model. (Sub-Hazard Ratios, 95% confidence intervals)

Mixed models will be used to model decline in kidney function. Random intercepts and random slopes will be used to account for individual variation in trajectories. As persons developing ESRD are more likely to miss subsequent follow up visits, we will impute estimated GFR as 15 ml/min/1.73m<sup>2</sup> at the development of ESRD as done previously.<sup>12</sup>

Potential covariates for multivariable regression models include: age, sex, percentage of African ancestry, body mass index, estimated glomerular filtration rate, cholesterol, systolic blood pressure, anti-hypertensive medication use, diabetes status, cardiovascular disease status, and smoking status.

**Limitations:** As this is an observational study, we cannot make causal inferences, and there may be residual confounding.

**7. a. Will the data be used for non-CVD analysis in this manuscript?**  Yes  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 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  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”?**  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.csc.unc.edu/ARIC/search.php>**

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)?**

Proposal #2981, “*APOLI* renal-risk variants, cardiovascular disease, and all-cause mortality in African-Americans” Morgan Grams

**11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?**  Yes  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)\* 2009.16; 2015.26)

\*ancillary studies are listed by number at <http://www.csc.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.**

**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.csc.unc.edu/aric/index.php>, under Publications, Policies & Forms.

[http://publicaccess.nih.gov/submit\\_process\\_journals.htm](http://publicaccess.nih.gov/submit_process_journals.htm) shows you which journals automatically upload articles to PubMed central.

**13. Per Data Use Agreement Addendum, approved manuscripts using CMS data shall be submitted by the Coordinating Center to CMS for informational purposes prior to publication.** Approved manuscripts should be sent to Pingping Wu at CC, at [pingping\\_wu@unc.edu](mailto:pingping_wu@unc.edu). I will be using CMS data in my manuscript  Yes  No.

## References

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10. Grams ME, Surapaneni A, Ballew SH, Appel LJ, Boerwinkle E, Boulware, et al. *APOLI* kidney-risk variants and cardiovascular disease: An individual participant data meta-analysis. *In press*.
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