1.a. Full Title: Mitochondrial Copy Number and ApoL1 Risk Allele Status in the Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters): ApoL1 and mtDNA Copy Number

2. Writing Group:
   Avi Z. Rosenberg MD, PhD, Adrienne Tin PhD, Jeffrey B. Kopp MD, Josef Coresh, MD, PhD, Dan E. Arking PhD, Morgan Grams MD, PhD, others welcome

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

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3. Timeline:
   Data analysis will start immediately. A manuscript is expected to be prepared within 6 months.

4. Rationale:
African-American populations are at increased risk for cardiovascular events and chronic kidney disease. Two variant haplotypes in the Apolipoprotein L1 (APOL1) gene have been described in those of African descent that are associated with a predisposition to focal segmental glomerulosclerosis (FSGS) and more recently demonstrated to contribute to increased cardiovascular risk and CKD in general (Ito et al.; Saab et al.). The mechanism of injury associated with APOL1 risk allele expression is not clear, though some data suggests a role in vascular injury (Madhavan et al.). We hypothesize that one mechanism of APOL1-associated injury is mitochondrial mediated and herein propose a first population-based study to test.

In light of the recently reported utility of mitochondrial copy number as an index of overall mortality and frailty (Ashar et al.), we propose to explore a possible relationship between APOL1 risk allele status and mitochondrial copy number. A role for autophagic and mitochondrial dysfunction in FSGS is being elaborated (i.e. (Kawakami et al.)). Relevant to our study is data showing ApoL1 interactions with cardiolipin (Wan et al.; Zhaorigetu et al.). More recently, data has suggested an interaction of an ApoL1 splice variant that is present in mitochondria and alters mitochondrial function (unpublished, J.B. Kopp). Thus, we propose to assess whether APOL1 risk allele status correlates with mitochondrial injury as assessed by mtDNA copy number variation. Furthermore, whereas mtDNA copy number correlated with all cause mortality in a age/sex, race stratified analysis, we propose to test whether APOL1 can further stratify the African-American cohort thereby providing added insight into the progression to chronic kidney disease end stage renal disease in ARIC as well as potential mechanistic insight into these entities.

5. Main Hypothesis/Study Questions:

1. APOL1 risk allele status will correlate with mitochondrial copy number.

2. Mitochondrial copy number will partially mediate the relationship between APOL1 and end-stage renal disease.

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: APOL1-risk allele stratified meta-analysis

Inclusion/exclusion: African American ARIC participants with APOL1 genotyping, to include G0, G1a, G1 and G2 allele. Persons with ESRD at baseline will be excluded.

Outcome: mtDNA copy number, the latter assessed as reported by Ashar et al 2015 (array-derived).
**Predictor:** *APOL1* high risk status, defined as the presence of two risk alleles, as well as the following 5 combination of individual risk alleles: G0/G0, G0/G1, G1/G1, G1/G2, G2/G2.

**Covariates:** Age, sex, eGFR, history of diabetes, history of hypertension, coronary heart disease and vascular disease.

**Data analysis:** The distribution of mtDNA copy number will be compared across *APOL1* status. The association between mtDNA copy number and *APOL1* status will be evaluated using linear regression, adjusting for age, race, sex, and eGFR. In a second model, additional adjustments will be made for baseline hypertension, diabetes, coronary heart disease, and peripheral vascular disease. If there is significant association between *APOL1* and mtDNA copy number, we will evaluate for mediation between *APOL1* high-risk status and incident end-stage renal disease using multivariable Cox proportional hazards regression. This will be implemented by fitting the model with and without mtDNA and comparing the coefficients on *APOL1* high-risk status.

**Limitations:**
1. The number of African-American patients with *APOL1* genotyping is limited. If this study demonstrates an *APOL1*-associated signal, additional cohorts can be explored.
2. The data available utilizes mtDNA derived from peripheral blood. ApoL1 mediated injury may impact mitochondrial DNA copy number in an organ/cell-type-specific manner.

**7.a. Will the data be used for non-CVD analysis in this manuscript?**  
___X___ 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?**  
___X___ 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?**  
___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

**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)?

2306 The role of mitochondrial copy number and genetic variation in mortality
2003 Associations of APOL1 variants with microvascular and cardiovascular disease in African Americans

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*2006.03, 2007.02, 2013.23)
 ___  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.

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.
References


