ARIC Manuscript Proposal #2519

PC Reviewed: 4/14/15	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: **Mitochondrial Copy Number and Kidney Outcomes in the Atherosclerosis Risk in Communities (ARIC) Study**

b. Abbreviated Title (Length 26 characters): **mtDNA copy number and the kidney**

2. Writing Group:
Adrienne Tin, Morgan Grams, Avi Z. Rosenberg, Foram Ashar, Josef Coresh, Dan E. Arking, and others welcome

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

F**irst author**: **Adrienne Tin, PhD**
Address: Department of Epidemiology
Johns Hopkins Bloomberg School of Public Health
615 N. Wolfe Street, W6021
Baltimore, MD 21205
Phone: 201-281-9577
atin1@jhu.edu

ARIC author to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).
Name: **Dan Arking, PhD**
Address: Miller Research Building, Room 447
733 N. Broadway St.
Baltimore, MD 21205
Phone: (410) 502-4867
Fax: (410) 614-8600
arking@jhmi.edu

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

4. Rationale:
Mitochondria are organelles that play an important role in the energy synthesis of cells. Reduced mitochondrial DNA (mtDNA) copy number in peripheral blood has been

associated with risk factors of chronic kidney disease (CKD), including diabetes and obesity[1, 2] and linked to oxidative stress.[3] Higher levels of inflammation biomarkers, which can be induced by oxidative stress, have been associated with kidney function decline.[4-6] It is unknown whether reduced mtDNA copy number will be associated with kidney function decline independent of known risk factors of CKD.

5. Main Hypothesis/Study Questions:

Lower mtDNA copy number will be associated with adverse kidney outcomes, including incident ESRD, CKD, kidney failure, and acute kidney injury (AKI), independent of known risk factors of CKD.

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: prospective cohort study

Inclusion criteria: Participants who consented to genetic study with available data on mtDNA copy number (i.e. having been run on the Affymetrix genotyping array), outcome variables, and covariates.

Outcomes: incident ESRD, CKD, kidney failure, and acute kidney injury

Predictor: Relative mtDNA copy number derived from A/B allele intensity of the mitochondrial SNPs (n=119) in Affymetrix GWAS microarray. More detailed methods were described in ARIC proposal #2335. Briefly, haploidy was assumed for all mitochondrial SNPs. The median probe intensity difference between the A/B alleles across all mitochondrial SNPs is taken as a measure of the relative mtDNA copy number for each sample. In addition, principal components (PC) on probe intensities for both A/B alleles were generated from a randomly chosen subset of 10,000 autosomal SNPs. The relative mtDNA copy number is the residuals of the median probe intensity difference adjusted for the first 20 PCs, age, sex, and study center.

Other variable of interest: age, diabetes, hypertension, eGFR, BMI, white blood cell count, serum albumin at the visit where blood samples were used for the assay of GWAS microarray, gender and race group.

Data analysis:
Relative mtDNA copy number will be categorized by quartiles.
We will use Cox regression to test for the association between relative mtDNA copy number levels and kidney outcomes. The proportional hazard assumption will be examined using time interaction term and plots of the estimated cumulative hazards.
Limitations:

1. The relative mtDNA copy numbers were derived from GWAS microarray data from peripheral blood.
2. No albuminuria.

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)?

#2335 mtDNA copy number GWAS
#1949 Validation of inter-visit kidney events

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 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

1. Lee HK, Song JH, Shin CS, et al. Decreased mitochondrial DNA content in peripheral blood precedes the development of non-insulin-dependent diabetes mellitus. *Diabetes Res Clin Pract.* 1998;42:161-167.
2. Lee JY, Lee DC, Im JA, Lee JW. Mitochondrial DNA copy number in peripheral blood is independently associated with visceral fat accumulation in healthy young adults. *Int J Endocrinol.* 2014;2014:586017.
3. Liu CS, Tsai CS, Kuo CL, et al. Oxidative stress-related alteration of the copy number of mitochondrial DNA in human leukocytes. *Free Radic Res.* 2003;37:1307-1317.
4. Shankar A, Sun L, Klein BE, et al. Markers of inflammation predict the long-term risk of developing chronic kidney disease: a population-based cohort study. *Kidney Int.* 2011;80:1231-1238.
5. Hiramoto JS, Katz R, Peralta CA, et al. Inflammation and coagulation markers and kidney function decline: the Multi-Ethnic Study of Atherosclerosis (MESA). *Am J Kidney Dis.* 2012;60:225-232.
6. Bash LD, Erlinger TP, Coresh J, et al. Inflammation, hemostasis, and the risk of kidney function decline in the Atherosclerosis Risk in Communities (ARIC) Study. *Am J Kidney Dis.* 2009;53:596-605.