ARIC Manuscript Proposal #2306

PC Reviewed: 2/11/14 **Status: A** **Priority: 2**
SC Reviewed: _____ **Status: _____** **Priority: ____**

1.a. Full Title: The role of mitochondrial DNA copy number and genetic variation in mortality

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

2. Writing Group:
Jeremy Walston
Foram Ashar
Megan Grove
Eric Boerwinkle
Additional interested ARIC investigators

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

First author: **Foram N. Ashar**
Address: 733 N Broadway, BRB 515
Baltimore, MD 21205

Phone: (443) 287-0251 Fax: (410) 614-8600
E-mail: fashar1@jhmi.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 E. Arking**
Address: 733 N Broadway, BRB 447
Baltimore, MD 21205

Phone: (410) 502-4867 Fax: (410) 614-8600
E-mail: arking@jhmi.edu

3. Timeline: First manuscript is already drafted. The CHD analyses are on-going, and we anticipate a manuscript will be ready by the end of summer 2014.

4. Rationale: Energy metabolism has long been hypothesized to play a role in human disease and aging. We have shown association of a mitochondrial allele variation

in the D-loop hypervariable region of the mitochondrial genome with the frailty phenotype in older adults (Moore et al., 2010). Given the central role of the D-loop in replication and maintenance of the mitochondrial genome, we hypothesized that this associated variant is likely to affect mitochondrial stability and copy number of the mitochondrial genome, and thereby influence the frailty phenotype and successful aging. Preliminary analyses in ~4,000 European ancestry individuals from the Cardiovascular Health Study (CHS) indicate that mtDNA copy number is significantly associated with baseline age ($P=1.94x10^{-6}$), and is a strong predictor of overall mortality (RR=0.85, 95%CI 0.80-0.89, $P=4.23x10^{-11}$), after adjustment for age. We further observed a strong association with baseline CHD ($P=4.77x10^{-10}$). The goals of the proposed study are to validate these results in a larger cohort, explore the role of mtDNA copy number on CHD risk factors, and expand results to include African Americans.

5. Main Hypothesis/Study Questions:
We hypothesize that mtDNA copy number will be a significant predictor of overall mortality in ARIC, as well as incident CHD and specific CHD risk factors.

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).
Initial analyses will rely upon existing genotyping microarray data to extract mtDNA copy number, and all variables used will be drawn from the visit at which the DNA was collected. Given the effect sizes in CHS, we are fully powered to observe an effect on mortality and incident CHD in ARIC whites. We propose using the entire cohort to allow us to perform sub-group analyses, and to look for association between mtDNA and specific CVD risk factors (e.g. inflammatory markers, HTN, DM, lipids, etc..). We also propose looking in blacks, to see if the effects are replicable across ethnic groups.

Assuming positive results with initial analyses, we propose to look at longitudinal changes in mtDNA copy number, by examining DNA isolated from different visits. Specifically, we are using qPCR to assess mtDNA copy number from DNA obtained at visit 1 (see AS 2013.23), and will compare that to results obtained from microarrays, which are drawn mostly from visits 2 and 3. We will compare baseline effects to change in mtDNA copy number over time, and explore whether using mtDNA copy number measurements closer to the incident event has greater predictive utility.

Exploratory data analyses with individuals stratified into quintiles based on mtDNA copy number will incorporate standard descriptive statistics and graphing methods to represent baseline demographic, anthropometric, and disease related characteristics of the study population, and independent variables of interest, as well as baseline CHD and mortality. We anticipate needing to adjust quintiles for age (using age at DNA collection) and sex based on findings in CHS.

Survival analyses methods will be employed to evaluate potential differences in the incidence of CHD and mortality by mtDNA copy number (treated as a continuous quantitative trait). After model construction steps, to accommodate the survival outcome and potential for sex specific differences, we anticipate that final models will incorporate age, sex, and be stratified on self-reported race to address population substructure. The

magnitude, direction, and statistical significance of coefficients will be assessed. We will also examine the relationship between mtDNA copy number and CHD and mortality, incorporating known CHD risk factors, as part of a mediation analysis to see whether mtDNA copy number is acting through specific pathways. Similar analyses will be performed using longitudinal measures of mtDNA copy number. Additionally, we will explore using change in mtDNA copy number, as well as using measures from multiple DNA isolations (using liner mixed models).

For genetic associations, mtDNA copy number will be treated as a continuous quantitative trait, and association with specific DNA variants and mitochondrial haplogroups will be determined using a linear regression frame-work, with mtDNA copy number as the dependent variable. Covariates will include age, sex and BMI. For each haplogroup, we will compare that haplogroup vs. all others combined, and use permutation to account for multiple testing.

To address the possibility that observed results may be a consequence of unique characteristics of the data or subgroups within the population a series of sensitivity analyses will be conducted after each phase of analysis. Stratification by levels of physical activity, diseases not previously excluded with suggested relationships to mitochondria such as diabetes and Parkinson's disease (Wallace DC, 2005), and by use of medications that may impact muscle strength such as ACE inhibitors (Di Bari M, van de Poll-Franse LV et al., 2004) will be explored. Additional analyses will explore cause specific mortality as well as addressing concerns regarding, interview mode, and missing data.

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)?

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:

Burke, Gregory L., Alice M. Arnold, Diane E. Bild, Mary Cushman, Linda P. Fried, Anne Newman, Cathy Nunn, and John Robbins. "Factors associated with healthy aging: the cardiovascular health study." Journal of the American Geriatrics Society 49, no. 3 (2001): 254-262.

Chen, X. J., & Butow, R. A. (2005). The organization and inheritance of the mitochondrial genome. *Nat Rev Genet, 6*(11), 815-825. doi:10.1038/nrg1708

De Benedictis G, Rose G, Carrieri G, De Luca M, Falcone E, Passarino G, Bonafe M, Monti D, Baggio G, Bertolini S, Mari D, Mattace R, Francheschi C. Mitochondrial DNA inherited variants are associated with successful aging and longevity in humans. FASEB J. 1999. Sep; 13(12), 1532-6.

Di Bari M, van de Poll-Franse LV, Onder G, Kritchevsky SB, Newman A, Harris TB, et al. Antihypertensive medications and differences in muscle mass in older persons: The health, aging and body composition study. J Am Geriatr Soc. 2004 Jun;52(6):961-6

Moore, AZ, Biggs, M.L., Mattein, A., O'Connor, A., McGuire, S., Beamer, B.A., Fallin, M.D., Fried, L.P., Walston, J., Chakravarti, A., Arking, D.E. (2010). Polymorphisms in the mitochondrial DNA control region and frailty in older adults. *PLoS One*, 5(6):e11069.

Newman, Anne B., Alice M. Arnold, Barbara L. Naydeck, Linda P. Fried, Gregory L. Burke,
Paul Enright, John Gottdiener, Calvin Hirsch, Daniel O'Leary, and Russell Tracy. "" Successful aging": effect of subclinical cardiovascular disease." Archives of internal medicine 163, no. 19 (2003): 2315.

Ruggiero C, Ferrucci L. The endeavor of high maintenance homeostasis: Resting metabolic rate and the legacy of longevity. J Gerontol A Biol Sci Med Sci. 2006 May;61(5):466-71.

Scheffler IE. Mitochondria. 2nd ed. Hoboken, New Jersey: John Wiley & Sons, Inc.; 2008. Suomalainen, A., & Isohanni, P. (2010). Mitochondrial DNA depletion syndromes – Many genes, common mechanisms. *Neuromuscular Disorders*, *20*(7), 429-437. doi:10.1016/j.nmd.2010.03.017

van der Walt JM, Nicodemus KK, Martin ER, Scott WK, Nance MA, Watts RL, Hubble JP, Haines JL, Koller WC, Lyons K, Pahwa R, Stern MB, Colcher A, Hiner BC, Jankovic J, Ondo WG, Allen FH Jr, Goetz CG, Small GW, Mastaglia F, Stajich JM, McLaurin AC, Middleton LT, Scott BL, Schmechel DE, Pericak-Vance MA, Vance JM. Mitochondrial polymorphisms significantly reduce the risk of Parkinson disease. Am J Hum Genet. 2003 Apr;72(4):804-11. Epub 2003 Feb 28.

Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: A dawn for evolutionary medicine. Annu Rev Genet. 2005;39:359-407.