ARIC Manuscript Proposal # 3160

PC Reviewed: 5/8/18	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Association between Mitochondrial DNA Copy Number and heart failure: Findings from the Atherosclerosis Risk in Communities Study (ARIC)

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

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

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3. Timeline: We anticipate a manuscript will be ready by the end of summer 2018.

4. Rationale:

Heart failure (HF) is a public health problem affecting 23 million people globally and 5.7 million people in the United States alone.[1,2] It is also the most rapidly growing cardiovascular disease worldwide.[3] The prevalence of HF in the US is expected to increase by 46%, to 8.5 million by 2030, and its associated cost is expected to increase nearly 127% from $30.7 billion to $69.7 billion.[4] Despite some decline in mortality in the early 2000s due to improvements in treating and preventing heart failure, a recent community-based study showed a striking 5-year mortality of 52.6% after diagnosis.[5] Thus, it is critical to understand the disease mechanism to prevent and treat heart failure more effectively.

Mitochondria play a central role in energy production by utilizing glucose, other nutrients and oxygen to generate the energy-carrying molecule, adenosine triphosphate (ATP).[6] Unlike other organelles, mitochondria have their own circular DNA (mtDNA), which contains genetic coding information for proteins essential to the oxidative phosphorylation process. Each mitochondrion has 2 to 10 copies of mtDNA, leading to 10^3 to 10^4 copies of mtDNA per cell. The number of mtDNA copies (mtDNA-CN) depends on the size and number of mitochondria, which change under different energy demands and different degrees of oxidative stress.[7]

Decreased mtDNA-CN is a marker of mtDNA dysfunction, which is related to cardiovascular phenotypes including atherosclerosis, arrhythmias, and sudden cardiac death[8-10] as well as their risk factors including hypertension, diabetes, and chronic kidney disease.[11-14] In animal studies, mtDNA damage and depletion of mtDNA were associated with the development of dilated cardiomyopathy and impaired remodeling after ischemic injury.[15-18] In small-sample case-control studies, depletion of mtDNA in heart tissue samples was associated with heart failure.[19,20] A cohort study with 2-year follow-up also showed that heart failure patients with lower mtDNA-CN had more cardiovascular deaths and rehospitalization events than those with higher mtDNA-CN.[11]

However, the association between mtDNA-CN and incident HF has not been evaluated in a cohort of long-term follow-up and the effect of mtDNA-CN on incident HF in the general population is unknown. In the present study, we aim to examine the association between baseline mtDNA-CN and the risk of HF among participants from the Atherosclerosis Risk in Communities (ARIC) study.

5. Main Hypothesis/Study Questions:

We hypothesize that mtDNA-CN will be a significant predictor of incident heart failure, both HFpEF and HFrEF.

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 study.

Inclusion/exclusion: The study will include White and Black adults with data available for mtDNA-CN estimation. We will exclude non-whites and non-blacks, as well as participants with prevalent HF at the time of mtDNA-CN measurements, and participants missing HF data or other relevant covariates.

Measurement of HF events

Since ARIC adjudicates HF cases after 2005 and this project will use mtDNA-CN from samples predominantly collected before 2005, this project will define HF based on ICD codes. The details of this approach in the ARIC study has been described previously.[21] Briefly, HF events were identified using 1) annual phone calls and interviews about interim hospitalizations; 2) local hospital records of hospital discharges with cardiovascular diagnoses; and 3) death certificates from health departments. Any listing of an International Classification of Diseases (ICD)-9 code for HF on a hospital discharge sheet (428.0 to 428.9) or ICD-9 428.X or ICD-10 I50 on a death certificate were defined as HF. There was excellent agreement between codes from hospital discharge records in ARIC and those from Medicare claims (Kappa coefficient 0.92 for codes in any position and 0.80 for codes in primary position).[22]

The date of HF incidence will be defined as the date of first hospital discharge with HF codes, or when HF was listed as a cause of death, whichever came first. We will also evaluate HFrEF and HFpEF cases separately.

Measurement of mtDNA Copy Number

DNA samples were isolated from buffy coat and genotyped on the Affymetrix Genome-Wide Human SNP Array 6.0.[12,23] The median mitochondrial probe intensity across 25 high quality mitochondrial SNPs were used to determine raw mtDNA-CN. Technical artifacts, batch effects, DNA quality, and starting DNA quantity were corrected for by using surrogate variables (SV) generated from probe intensities from a set of 43,316 high quality autosomal SNPs.[24] We calculated residuals using a linear regression model with raw mtDNA-CN as the dependent variable and the first 15 SVs, age, sex, and enrollment center as the independent variables. The calculated residuals were then used as measurement for mtDNA-CN for all subsequent analyses.

Statistical Analyses

DNA for mtDNA-CN analysis was collected in visit 1 (1987-1989) for 492 participants (4.3%), visit 2 (1990-1992) for 9,152 participants (79.3%), visit 3 (1993-1995) for 1,796 participants (15.6%), visit 4 (1996-1998) for 67 participants (0.6%) and visit 5/MRI for

27 participants (0.2%). The baseline visit will be the visit of DNA collection for each participant and the covariates obtained from this visit will be used as the baseline information. Follow-up for incident HF events will be from the baseline visit until the incident event, or until December 31, 2014, whichever comes first.

Baseline characteristics of the study population will be compared across quintiles of mtDNA-CN. In the primary analysis, we will categorize mtDNA-CN into quintiles based on the sample distribution. We will use a Cox proportional hazards model and estimate the hazard ratios (HR) and 95% confidence intervals (CI) comparing the 2^{nd} to 5^{th} quintiles of mtDNA-CN to the 1^{st} quintile of mtDNA-CN, respectively. We will also test for a linear trend of HR across quintiles of mtDNA-CN. In the secondary analysis, the association between mtDNA-CN and incident HF will be estimated using mtDNA-CN as a continuous variable, by comparing the 10^{th} to the 90^{th} percentile of mtDNA-CN. Additionally, we will model mtDNA-CN as restricted cubic splines with knots at the 5^{th}, 35^{th}, 65^{th} and 95^{th} percentiles of its distribution to provide a smooth yet flexible description of the dose-response relationship between mtDNA-CN and HF.

We will use 3 models with progressive degrees of adjustment to adjust for potential confounders.

Model 1: Age, sex, race, and enrollment center
Model 2: Model 1 + body mass index, smoking, alcohol intake, physical activity
Model 3: Model 2 + total and HDL cholesterol, medication for dyslipidemia, systolic blood pressure, medication for hypertension, serum fasting glucose, diabetes, medication for diabetes, and CHD.

Subgroup analysis with pre-defined subgroups will be performed and tested for potential interactions. The subgroups will be: race, sex, prevalence of CVD at baseline, dyslipidemia, hypertension, and diabetes. We will also perform sensitivity analyses by excluding participants with prevalent CVD at baseline and by excluding participants from visit 5 because the treatment pattern may be distinct from the previous visits. In addition, the same analysis will be repeated with additional adjustment for log-transformed white blood cell count, which has been found to be associated with mtDNA-CN obtained from peripheral blood. Separate analyses will also be conducted for HFrEF and HFpEF cases.

All statistical analyses will be performed using Stata version 15 (StataCorp LP, College Station, Texas).

7.a. Will the data be used for non-CVD analysis in this manuscript? ____ Yes __ X__ 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?
__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? ____ 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.

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