**ARIC Manuscript Proposal #2772**

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| **PC Reviewed: 6/7/16** | **Status: A** | **Priority: 2** |
| **SC Reviewed: _____** | **Status: _____** | **Priority: ____** |

**1.a. Full Title**: Genetic variants of iron metabolism, arterial hypertension, and cardiac structure and function in the ARIC Study

**1.b. Abbreviated Title**: Genetics of iron metabolism and cardiovascular disease

**2. Writing Group**:
Writing group members: Odilson Marcos Silvestre, Sara B. Seidelmann, Miguel Morita Fernandes-Silva, Brian Claggett, John H. Eckfeldt, James S. Pankow, Eric Boerwinkle, Scott D. Solomon, others welcome

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

**First author**: **Odilson Marcos Silvestre**
Address: Brigham and Women's Hospital
Cardiovascular Division
75 Francis Street, PBB-1 North
Boston, MA 02115
Phone: 617-732-6575 Fax: 617-582-6027
E-mail: oms087@mail.harvard.edu OR odilsonsilvestre@yahoo.com.br

**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: **Scott D. Solomon**
Address: Brigham and Women's Hospital
Cardiovascular Division
75 Francis Street
Boston, MA 02115

Phone: 857-307-1960 Fax: 857-307-1944
E-mail: ssolomon@rics.bwh.harvard.edu

**3. Timeline**: Analysis will begin following proposal approval with the aim of completing analysis and a manuscript within 6 months.

**4. Rationale**:

Iron overload has been associated with cardiovascular disease. The accumulation of iron inside the cell releases free radicals, causing oxidant-mediated cellular injury and development of cardiomyopathy,[1] eventually resulting in HF. Likewise, iron overload can cause peroxidation of LDL cholesterol in the arteries leading to atherosclerotic lesions resulting in coronary disease. Although hemochromatosis, the main cause of iron overload, might lead to HF[2] and coronary disease,[3] it is still unclear whether genetic variants that predispose to the development of hemochromatosis are associated with cardiovascular consequences as arterial hypertension, and cardiac structural and functional changes in the general population.

Hereditary hemochromatosis is a leading cause of iron overload and is most commonly caused by mutations in the HFE (High Fe) gene. Three main variants have been associated with iron overload phenotype: rs1800562 (C282Y), rs1799945 (H63D), and rs1800730 (S65C). An analysis from the ARIC Study population found that about 37% of the white population were carriers or homozygotes for the C282Y or H63D variants. Although the phenotypic penetrance of these variants is low, they are associated with higher levels of ferritin or transferrin saturation among carriers than non-carriers in the general population.[4] As ferritin levels, reflecting iron stores, are independent predictors of vascular damage,[5] we hypothesize that HFE genes might be associated with a higher incidence of hypertension and changes in cardiac structure and function. The ARIC Study has a large bi-racial population with data on HFE mutation and long-term follow-up with validated adjudication of incidence of arterial hypertension and also echocardiography measures at Visit 5, being a high quality cohort to evaluate the association between HFE mutation, incident hypertension, cardiac structure and function and cardiovascular disease.

**Aim:**

We propose to test the association between genes variants of iron metabolism and risk of arterial hypertension, and cardiac structure and function assessed by echocardiography.

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

Gene variants that alter iron metabolism are associated with a higher risk of arterial stiffness resulting in hypertension, and changes in cardiac structure and function in an elderly population.

**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 methodological limitations or challenges if present).**

We will include individuals who were tested for HFE gene mutations at Visit 1 and will evaluate the association with incident arterial hypertension and arterial stiffness dynamically, across all five visits, as well as the association with echocardiographic parameters at Visit 5. We will exclude participants without information regarding HFE mutation.

**Variables to be evaluated**

**Exposures variables:**

rs1800562 (C282Y), rs1799945 (H63D), and rs1800730 (S65C) genotypes

**Outcome variables:**

- Incidence of arterial hypertension (Visits 1 to 5), pulse pressure (Visits 1 to 5), pulse wave velocity and arterial elastance
- Left ventricular dimensions, volume, systolic function evaluated by ejection fraction, LV diastolic measures, LA dimensions, tissue Doppler and speckle tracking based strain (longitudinal, circumferential and radial), RV dimension, volumes, and function.
- Incident heart failure, coronary disease, stroke, and all cause mortality.

**Other covariates:**

- Demographic characteristics (age, race, sex, and ARIC center)
- Cardiovascular risk factors (diabetes mellitus, dyslipidemia, systolic and diastolic blood pressure, body mass index, and smoking status)

**Analytical approach:**

Categorical data will be reported as percent frequencies and compared by chi-squared or Fischer exact tests. Mean and standard deviation will be used to display continuous normally distributed data. Non-normally distributed data will be displayed as median and $25^{th}$ -$75^{th}$ percentile. The association of HFE variants with hypertension will be analyzed using logistic regression models. We will create a univariate and a multivariable model to identify both the unadjusted and

adjusted risk of the outcomes of interest. Incident HF, coronary disease, stroke and death will be calculated and presented as events per 1000 person-years at risk. Analysis of genetic variants and cardiovascular events and death will be performed using Cox proportional hazards model. We will test for interaction for age and sex on the HFE mutation and cardiovascular outcomes. If sustained by a significant interaction, we will perform a stratified analysis by age and sex strata. We will also test for interaction considering HFE genes as an effect modifier on the association between cardiovascular risk factors (such as hypertension, diabetes, and dyslipidemia) and cardiovascular events. The association between HFE mutation, measures of arterial stiffness and echocardiography outcomes will be analyzed with multivariable linear regression controlling for potential confounders as: age, sex, BMI, ARIC center, arterial hypertension, diabetes mellitus, dyslipidemia, and smoking status. We will use visit 1 as baseline for this analysis. P-values <0.05 will be considered significant.

**Limitations:**

The association between HFE mutation and arterial hypertension, echocardiographic parameters, and cardiovascular disease will be analyzed without data about iron status, limiting the information in terms of phenotypic presentation.

**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 ICTDER03 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)?**

**#1986**: Adrienne Tin, Linda Kao, Joe Coresh, Meredith Atkinson, James Pankow, Eric Boerwinkle. Associations of hereditary hemochromatosis risk variants in the *HFE* gene with incident ESRD.

# 1083: John Eckfeldt, Eric Boerwinkle, Paul Adams, Eliseo Guallar, Jason Rogowski. Natural history of HFE-related hereditary hemochromatosis.

#2481: Iron status and incidence of cardiovascular events. The ARIC study. Odilson Marcos Silvestre, Alexandra Gonçalves, Brian Claggett, Wilson Nadruz Junior, Scott D. Solomon.

**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)*** #946)

*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. Fleming, R. E. & Ponka, P. Iron overload in human disease. *N. Engl. J. Med.* **366,** 348–359 (2012).
2. Gujja, P., Rosing, D. R., Tripodi, D. J. & Shizukuda, Y. Iron overload cardiomyopathy: better understanding of an increasing disorder. *J. Am. Coll. Cardiol.* **56,** 1001–1012 (2010).
3. Rasmussen, M. L. *et al.* A prospective study of coronary heart disease and the hemochromatosis gene (HFE) C282Y mutation: the Atherosclerosis Risk in Communities (ARIC) study. *Atherosclerosis* **154,** 739–746 (2001).
4. Adams, P. C. *et al.* Hemochromatosis and iron-overload screening in a racially diverse population. *N. Engl. J. Med.* **352,** 1769–1778 (2005).
5. Valenti, L. *et al.* Serum ferritin levels are associated with vascular damage in patients with nonalcoholic fatty liver disease. *Nutr. Metab. Cardiovasc. Dis. NMCD* **21,** 568–575 (2011).