ARIC Manuscript Proposal #2286

PC Reviewed: 1/14/13	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: sRAGE, Inflammation, and Risk of Atrial Fibrillation in a Community-based Population

b. Abbreviated Title (Length 26 characters): sRAGE and Atrial Fibrillation

2. Writing Group:
Writing group members:

Mahmoud Al Rifai
Andrea L.C. Schneider
Alvaro Alonso
Nisa Maruthur
Christina M. Parrinello
Brad C. Astor
Ron C. Hoogeveen
Elsayed Soliman
Lin Chen
Christie M. Ballantyne
Marc K. Halushka
Elizabeth Selvin
Others welcome

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

First author: Mahmoud Al Rifai, MD
MPH Candidate, 2014
Johns Hopkins Bloomberg School of Public Health
Address: 615 N Wolfe St #5041, Baltimore, MD 21205

Phone: 347-471-7060 **Fax**: 410-955-0476
E-mail: malrifai@jhsph.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: Elizabeth Selvin, PhD, MPH
Associate Professor of Epidemiology and Medicine
Johns Hopkins Bloomberg School of Public Health
Address: 2024 E Monument St, Suite 2-600, Baltimore MD 21287

Phone: 410-614-3752 **Fax**: 410-955-0476
E-mail: lselvin@jhsph.edu

3. Timeline:

The data needed for this analysis are currently available; we plan to submit for publication within 1-year (by December 2014).

4. Rationale:

Advanced glycation end products (AGEs) are hypothesized to contribute to the pathophysiology of vascular disease in diabetes. [1] There is also evidence to suggest a possible role of AGEs in vascular disease in persons without diabetes. [2] The interaction of AGEs with their cell-bound receptors (RAGE) results in cellular activation with ensuing inflammatory processes and tissue injury. Soluble receptors of RAGE (sRAGE) compete with RAGE by acting as a decoy receptor and thereby prevent cellular activation. [3] sRAGE has been shown in a number of community-based studies to be strongly inversely associated with markers of inflammation such as C-reactive protein [4], TNF-α [5] and white blood cell count. [6] This inverse association is thought to be a result of decreased binding of AGEs with RAGE with resulting decreased inflammation.[3] Interestingly several recent studies have shown that sRAGE rises in the setting of acute illness (e.g. pneumonia), [7] surgical interventions, [8] and trauma. [9]

These studies suggest that inflammation may be a mediator of the association of sRAGE with long-term cardiovascular outcomes. A role for inflammation in development and progression of cardiovascular disease is well established. [10] Since sRAGE has been shown to be associated with decreased inflammation, it may also be associated with lower cardiovascular disease incidence. The association of sRAGE with decreased inflammation could help explain why sRAGE is inversely associated with cardiovascular disease incidence in community-based studies. A previous ARIC study has shown that persons with higher levels of sRAGE at baseline had lower incidence of coronary heart disease, diabetes, and mortality during long-term follow-up compared to those with lower levels of sRAGE. [4] More recent work in ARIC has shown that sRAGE is strongly and inversely associated with incident heart failure [Lazo et al MSP #2170, AHA-EPI abstract, manuscript in progress]. Another ARIC study found that sRAGE was positively associated with incident chronic kidney disease in crude models but not after adjustment for baseline kidney function, suggesting the association of sRAGE with kidney disease may be explained by its partial clearance by the kidney [Rebholz et al MSP #2192, AHA-EPI abstract, manuscript under review by ARIC Publications Committee]. In a prospective cohort study of Japanese adult men, high esRAGE (endogenous secretory sRAGE), but not sRAGE, was associated with lowered incidence of metabolic

syndrome.[11] In a cross-sectional study of Latino youth, sRAGE was inversely and independently associated with waist circumference, mean arterial pressure, and insulin resistance but positively associated with HDL cholesterol. [12]

Little is known about the association of sRAGE with atrial fibrillation (AF). A cross-sectional study showed that patients with AF had higher levels of sRAGE. [13] To our knowledge, there are no prospective studies of sRAGE with incident AF. Since inflammation is associated with increased risk of AF [14] and sRAGE is associated with lower inflammation, we hypothesize that persons with lower levels of sRAGE at baseline would have a higher incidence of AF during long-term follow-up compared to those with higher sRAGE levels, and this association may be partially mediated by inflammation (as assessed by CRP).

5. Main Hypothesis/Study Questions:

Aim 1: To characterize the association of sRAGE with baseline inflammatory status and change in inflammatory status (defined by C-reactive protein).

> Hypothesis 1: Lower sRAGE will be independently associated with higher baseline CRP and an increase in CRP over 6 years.

Aim 2: To characterize the association of sRAGE with incident atrial fibrillation.

> Hypothesis 2: Lower sRAGE will be associated with increased risk of atrial fibrillation.

Aim 3: To determine if the prospective association of sRAGE with atrial fibrillation is mediated by inflammation (defined by C-reactive protein).

> Hypothesis 3: The association of sRAGE and AF will be partially mediated by progression of inflammation as assessed by changes in CRP during the first 6 years of follow-up.

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 and Inclusion/Exclusion criteria:

This is a prospective cohort analysis using a random subcohort of ~1200 participants with normal kidney function (as per the original design of the parent case-cohort ARIC Ancillary Study), without prevalent AF or CHD, and with sRAGE levels measured at visit 2. Using the most recent follow-up data available (currently through December 2010), we will examine the association of baseline (visit 2) sRAGE with incident cases of atrial fibrillation in the cohort random sample. We will also examine the cross-sectional

and prospective association between sRAGE and CRP using CRP measurements from both visit 2 and visit 4 and the possible mediating effect of CRP on the association of baseline sRAGE with risk of AF.

Exposure:
sRAGE: sRAGE was measured in 2010 by ELISA (R&D Systems, Minneapolis, MN) using samples that had been stored since collection in 1990-1992. The intra-assay CV was 2.8% and the inter-assay CV was 9.6%. [4]

Outcomes:
1. Incident atrial fibrillation events occurring after visit 2. Incident AF will be defined using a standard ARIC definition: [15]

a. EKGs performed at study visits
b. Hospitalization discharge codes for AF or atrial flutter (ICD-9 codes 427.31 or 427.32) not accompanied by a code for cardiac surgery (ICD-9 codes 35.X or 36.X)
c. AF or Atrial Flutter listed as cause of death.

Note: Using the most recent follow-up data available (through December 2010), there have been 131 incident cases of atrial fibrillation (median follow-up period 18 years) in the cohort random sample.

2. High-sensitivity C-reactive protein.

a. Visit 2: CRP was measured in 2012-2013 in serum sample stored since collection in 1990-1992 using the Roche (Roche Modular P800 autoanalyzer) at the University of Minnesota. [4]
b. Visit 4: CRP was measured in 2010 in plasma samples stored since collection in 1996-1998 with a high sensitivity immunonephelometric assay on a BNII analyzer (Siemens Healthcare Diagnostics, Deerfield, Illinois) at the Baylor College of Medicine. The reliability coefficient for the hs-CRP assay at visit 4 was 0.99 (based on 421 blinded replicate samples). [16]

Note: A formal calibration study was conducted to compare measurements in serum at University of Minnesota to measurements in plasma at Baylor [Parrinello, MSP# 2243]. Among participants who had plasma available at all five visits, 200 participants were selected using stratified random sampling based on 5-year baseline age, gender and race/ethnicity. The ARIC Coordinating Center identified additional exclusions for participants who had events between visits. University of Minnesota results from visit 2 serum were compared to the previously obtained values from visit 4 plasma at Baylor, accounting for differences between specimen type, lab, and calendar time in a single regression. These analyses showed that CRP measured from frozen visit 4 plasma were comparable to CRP measured from frozen visit 2 serum and it was concluded that no calibration would be needed for CRP.

Covariates:

Age (years), sex (male; female), race-center (Minnesota whites; Maryland whites; North Carolina whites; North Carolina blacks; Mississippi blacks), smoking status (current; former; never), alcohol use (current; former; never), blood pressure medication use; systolic blood pressure and diastolic blood pressures (continuous variables, per mmHg),

total cholesterol (mg/dL), HDL cholesterol (mg/dL), diabetes status (self-reported history, medication use, fasting glucose ≥126 mg/dl, HbA1c ≥6.5%), estimated glomerular filtration rate, and BMI (kg/m^2). We will also explore the possibility of adjusting for the CHARGE-AF risk score, a predictive model for AG that includes all major clinical risk factors for AGE (derived in ARIC, CHS, and Framingham). [17]

Statistical Analysis:
We will analyze sRAGE both continuously and categorized in quartiles. In analyses using quartiles, the highest quartile (Q4) will be the reference category. Baseline characteristics of the population will be presented overall and by sRAGE quartile.

Aim 1:
We will study the cross-sectional and prospective associations between baseline sRAGE and CRP using regression models before and after adjustment for covariates. We will use logistic regression to evaluate the cross-sectional association between baseline sRAGE and baseline high CRP (defined as ≥3 mg/L) versus normal/moderate CRP (<3 mg/L). For our prospective analyses among the subsample of persons who also attended visit 4, we will use adjusted multinomial logistic regression to look at the association between baseline sRAGE and categories of CRP change:

CRP <3 mg/L at both visits 2 and 4 (always normal/moderate)
CRP <3 mg/L at visit 2 and ≥3 mg/L at visit 4 (normal/moderate to high)
CRP ≥3 mg/L at visit 2 and <3 mg/L at visit 4 (high to normal/moderate)
CRP ≥3 mg/L at visit 2 and ≥3 mg/L at visit 4 (always high).

Aim 2:
We will study the incidence of AF in participants by quartiles of sRAGE using Kaplan Meier analyses. We will then use adjusted Cox proportional hazards models to estimate the hazard ratios (95% confidence intervals) for the association of baseline sRAGE with incident AF. The proportional hazards assumptions will be checked with the use of Schoenfeld residuals and graphic methods. We will also look at the association of sRAGE with incident AF continuously using linear splines with knots corresponding to each of the quartiles and restricted cubic splines with knots at 10^{th}, 50^{th}, and 90^{th} percentiles. [18]

We will use two main models for the above analyses:

Model 1: Adjusted for demographic factors: age, sex, race-center.

Model 2: Adjusted for demographic factors and cardiovascular risk factors: Model 1 + smoking status, alcohol use, blood pressure medication use, systolic blood pressure, diastolic blood pressure, total cholesterol, diabetes status, BMI.

We will conduct sensitivity analyses with additional adjustment for eGFR and analyses using the CHARGE-AF risk score and we will also test for effect modification by race.

Aim 3:
We will model CRP as a time-varying covariate (visit 2 and visit 4 CRP) in our Cox-proportional hazards models to estimate the hazard ratios (95% confidence intervals) for the association of baseline sRAGE with incident AF. We will compare the hazard ratios (95% confidence intervals) in models with and without adjustment for CRP to see if there is attenuation when CRP is added to the model (suggesting partial or full mediation by CRP). [16]

Limitations:

- We only have one measurement of sRAGE at visit 2. Although sRAGE is known to vary over time, the 3-year within-person variability in ARIC is similar to other standard risk factors such as cholesterol. [19]
- We have a relatively limited sample size because sRAGE was only measured for a subpopulation, therefore we may have limited power to detect moderate associations between sRAGE and AF. Nonetheless, we have extensive follow-up in all participants in the subcohort and there have been >100 cases of AF in this subsample.

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?
____ Yes _X_ 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"?
____ 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)?

1. Genetic basis of the black-white differences in sRAGE levels: Results from the Atherosclerosis Risk in Communities Study- Nisa Marthur (#2251)
2. sRAGE, progression of subclinical cardiac damage, and risk of heart failure- Mariana Lazo-Elizondo (#2170)
3. Determinants of sRAGE and its Association with Cardiovascular Disease, Diabetes, and Mortality in a Community-based Population (MS1890)- Selvin, E (#1890)
4. The Association of Lifestyle Factors with Circulating levels of the Soluble Receptor for Advanced Glycation End Products (sRAGE) (MS1905)- Bower, J. K (#1905)
5. Soluble RAGE and Risk of Kidney Disease Outcomes: the Atherosclerosis Risk in Communities (ARIC) Study- Casey Rebholz (#2192)
6. C-reactive protein and mortality in individuals with atrial fibrillation: the ARIC study- Hermida, J (#1665)
7. Associations of C-reactive protein over six years with incident diabetes, cardiovascular events and mortality- Parrinello, Christina (#2207)
8. Prediction of atrial fibrillation in the community: the CHARGE consortium- Alvaro, Alonso (#1578)

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*

Longitudinal Study of Predictors and Consequences of Chronic Kidney Disease, PI: Brad Astor: Study number 2006.16
Epidemiologic study of risk factors and biomarkers of atrial fibrillation, PI: Alvaro Alonso: Study number 2008.12
Short-term Markers of Glycemia and Long-term Outcomes, PI: Elizabeth Selvin: Study number 2009.16

___ 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. Goldin A, Beckman J, Schmidt AM, Creager M. Advanced glycation end products: Sparking the development of diabetic vascular injury. *Circulation*. 2006;114(6):597.

2. Uribarri J, Stirban A, Sander D, et al. Single oral challenge by advanced glycation end products acutely impairs endothelial function in diabeticand nondiabetic subjects. *Diabetes Care*. 2007;30(10):2579.

3. Selejan S, Pöss J, Hewera LM, Kazakov A, Böhm M, Link A. Role of receptor for advanced glycation end products in cardiogenic shock. *Critical Care Medicine*. 2012;40(5):1513.

4. Selvin E, Halushka M, Rawlings A, et al. sRAGE and risk of diabetes, cardiovascular disease, and death. *Diabetes*. 2013;62(6):2116.

5. McNair ED, Wells CR, Qureshi AM, et al. Modulation of high sensitivity C reactive protein by soluble receptor for advanced glycation endproducts. *Molecular and Ceullular Biochemistry*. 2010;341(1-2):135.

6. Haiying Z, Sadatomo T, Yoshiki S, et al. Role of soluble receptor for advanced glycation end products on endotoxin-induced lung injury

. *American Journal of Respiratory and Critical Care Medicine*. 2008;178(4):356.

7. Narvaez-Rivera RM, Rendon A, Salinas-Carmona MC, Rosas-Taraco AG. Soluble RAGE as a severity marker in community acquired pneumonia associated sepsis. *BMC Infectious Diseases*. 2012;12(15).

8. Shah RJ, Bellamy SL, Lee JC, et al. Early plasma soluble receptor for advanced GlycationEnd-product levels are associated with bronchiolitis obliterans syndrome. *American Journal of Transplantation*. 2013;13:754.

9. Cohen MJ, Carles M, Brohi K, et al.
Early release of soluble receptor for advanced glycation endproducts after severe trauma i n humans.. *Journal of Trauma-Injury Infection & Critical Care*. 2010;68(6):1273.

10. Yousuf O, Mohanty B, Martin S, et al. High-sensitivity C-reactive protein and cardiovascular disease: A resolute belief or an elusive link? *Journal of the American College of Cardiology*. 2013;62(5):397.

11. Momma H, Niu K, Kobayashi Y, et al. Lower serum endogenous secretory receptor for advanced glycation end product level as a risk factor of metabolic syndrome among japanese adult men: A 2-year longitudinal study. *J Clin Endocrinol Metab*. 2013.

12. Brickey SR, Ryder JR, McClellan DR, Shaibi GQ. Soluble receptor for advanced glycation end products independently predicts cardiometabolic syndrome in latino youth. *Pediatr Diabetes*. 2013.

13. Raposeiras-Roubín S, Rodiño-Janeiro B, Grigorian-Shamagian L, et al. Evidence for a role of advanced glycation end products in atrial fibrillation. *International Journal of Cardiology*. 2012;157(3):397.

14. Huxley R, Lopez F, MacLehose R, et al. Novel association between plasma matrix metalloproteinase-9 and risk of incident atrial fibrillation in a case-cohort study: The atherosclerosis risk in communities study. *Plos One*. 2013;8(3).

15. Alonso A, Lopez FL, Matsushita K, et al. Chronic kidney disease is associated with the incidence of atrial fibrillation: The atherosclerosis risk in communities (ARIC) study. *Circulation*. 2011;123(25):2946-2953.

16. Hermida J, Lopez F, Montes R, Matsushita K, Astor B, Alonso A. Usefulness of high-sensitivity C-reactive protein to predict mortality in patients with atrial fibrillation (from the atherosclerosis risk in communities [ARIC] study). *The American Journal of Cardiology*. 2012;109(1):95.

17. Alonso A, Krijthe BP, Aspelund T, et al. Simple risk model predicts incidence of atrial fibrillation in a racially and geographically diverse population: The CHARGE-AF consortium. *J Am Heart Assoc*. 2013;2(2):e000102.

18. Harrell FE. *Regression modeling strategies: With applications to linear models, logistic regression, and survival analysis* . First ed. Springer; 2002.

19. Bower JK, Pankow JS, Lazo M, et al. Three-year variability in plasma concentrations of the soluble receptor for advanced glycation end products (sRAGE). *Clin Biochem*. 2013.