

**ARIC Manuscript Proposal #2609**

**PC Reviewed:** 9/8/15  
**SC Reviewed:** \_\_\_\_\_

**Status:** A  
**Status:** \_\_\_\_\_

**Priority:** 2  
**Priority:** \_\_\_\_\_

**1.a. Full Title: Brain Natriuretic Peptide as a Mediator of Racial and Genetic Admixture Differences in Incident Atrial Fibrillation and Congestive Heart Failure**

**b. Abbreviated Title (Length 26 characters): BNP as Mediator in AF and CHF**

**2. Writing Group:**

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

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### 3. **Timeline:**

Dr. Marcus has this data available from previously approved manuscript proposals and grant funding. Isaac Whitman, the first author, is a PGY7 first year Electrophysiology fellow, with approximately one third of his time protected for research. After recently completing a yearlong post-doctoral clinical and translational research certificate in epidemiology and biostatistics, Dr. Whitman will have the knowhow and support from Dr. Marcus (a Master in Clinical Epidemiology) and the biostatistics department at UCSF to complete this project within one year of its approval. In addition, we already have certification from the UCSF Committee on Human Research to perform this study, as they do not require specific approval to analyze de-identified data.

### 4. **Rationale:**

AF is the most common cardiac arrhythmia in the United States and the leading cause of embolic stroke.<sup>1,2</sup> Hypertension and HF are established strong risk factors for development of AF.<sup>3,4</sup> However, despite the finding that these and other AF risk factors are more common in blacks,<sup>5-9</sup> it has recently been demonstrated that whites have a greater risk for AF,<sup>10-12</sup> as do blacks with greater European ancestry.<sup>13-15</sup> The pathophysiology underlying this paradox is unknown.

Blacks also have a substantially higher risk for HF than whites,<sup>6,16</sup> but the mechanism underlying this difference is similarly poorly understood.

Of interest, AF is associated with higher atrial natriuretic peptide (ANP) levels.<sup>17,18</sup> Evidence that genetic aberrations in the ANP gene are associated with AF<sup>19</sup> raises the question as to whether abnormalities in ANP expression may cause AF (rather than reflect an atrial myopathy secondary to AF). While ANP levels have not been measured in any large cardiovascular cohorts with a substantial number of black participants and ascertainment of AF, the related protein Nt-proBNP is measured in ARIC and the Cardiovascular Health Study (CHS). Serum levels of Nt-proBNP have been closely correlated with ANP,<sup>17,20-22</sup> have similarly been shown to predict incident AF,<sup>17,23</sup> and are known to have diuretic effects that might relieve volume overload in the setting of HF.<sup>24-26</sup> Recent evidence demonstrates that Nt-proBNP levels are lower in blacks.<sup>27,28</sup>

Therefore, it is plausible that Nt-proBNP levels might mediate the discordant race-AF and race-HF associations. Specifically, the higher level of natriuretic peptides in whites may have a direct atrial effect that increases AF susceptibility. Higher levels of Nt-proBNP, via the diuretic action of this protein, may also render whites less prone to HF.

The heterogeneous genetics of African Americans comprising both African and European ancestry also pose context to test the mechanistic hypothesis of Nt-proBNP in AF and HF. Among African Americans, more European ancestry has been shown to impose greater risk of incident AF compared to those with less.<sup>15</sup> As with the differential racial association with AF and HF, Nt-proBNP may mediate the differential incidence of AF among African Americans across degrees of European genetic admixture. Should there also exist a differential risk of HF based on proportion of European ancestry, Nt-proBNP may mediate that differential association as well.

Understanding these relationships might help to elucidate the mechanisms underlying both of these important diseases and may point to specific prediction strategies and novel treatment approaches informed by racial background.

We hypothesize that baseline Nt-proBNP levels mediate the racial differences between blacks and whites, and the genetic ancestral differences within blacks, in incident AF and HF in ARIC, and will perform similar analyses in CHS testing the same hypotheses.

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

**Aim 1:** To determine if Nt-proBNP mediates the racial differences between blacks and whites in incident AF.

**Hypothesis already established in ARIC and CHS<sup>10,12</sup>:** Whites will have a greater incidence of AF compared to blacks.

**Hypothesis already established in ARIC and other cohorts<sup>27,28</sup>:** Whites will have higher mean baseline Nt-proBNP levels compared to blacks.

**Hypothesis:** Nt-proBNP level will mediate the difference in incident AF between whites and blacks.

**Aim 2:** To determine if Nt-proBNP mediates the racial differences between blacks and whites in incident HF.

**Hypothesis already established in ARIC and other cohorts<sup>6,16,27</sup>:** Blacks will have a greater incidence of HF compared to whites.

**Hypothesis already established in ARIC and other cohorts<sup>27,28</sup>:** Blacks will have lower mean baseline Nt-proBNP levels compared to whites.

**Hypothesis:** Nt-proBNP level will mediate the difference in incident HF between blacks and whites.

**Aim 3:** To determine if Nt-proBNP mediates the European ancestral differences in blacks in incident AF.

**Hypothesis already established in ARIC and CHS<sup>15</sup>:** Blacks with greater European ancestry will have greater incidence of AF.

**Hypothesis:** Nt-proBNP levels will mediate the difference between European ancestry and incident AF among blacks.

**Aim 4:** To determine if Nt-proBNP mediates the European ancestral differences in blacks in incident CHF.

**Hypothesis 1:** Blacks with greater European ancestry will have lower incidence of CHF.

**Hypothesis 2:** Nt-proBNP levels will mediate the difference between European ancestry and incident CHF among blacks.

## **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).**

As noted above, we are proposing to perform a similar analysis in CHS. The datasets include baseline ascertainment of Nt-proBNP levels and longitudinal documentation of cardiac rhythm status. For each aim/hypothesis, we intend to perform separate analyses for each cohort (ARIC and CHS), followed by a combined analysis.

Data to be used in this study include the following (the majority of which Dr. Marcus has already from previously approved manuscripts proposals and grant funding):

- Visit 4 Demographic and Medical Data: age, sex, race, body mass index, and smoking status
- Prevalent and Incident Events: diabetes, hypertension, heart failure, coronary artery disease, myocardial infarction, chronic kidney disease, atrial fibrillation, and death
- Genetic Data: genotyping of rs2200733, rs2106261, rs6666258, rs3903239, rs3807989, rs10821415, rs10824026, rs1152591, rs7164883 (from the Affymetrix 6.0 array).

The only additional data we will need from ARIC to perform our analysis will be Nt-proBNP data from Visit 4 (1996-98), which is available for >10,000 patients in whom there are more than 1,000 AF events. This visit will serve as the baseline for our study.

We will conduct two separate analyses with AF and HF as respective outcomes. These analyses will mirror one another. Similarly, while the primary predictor will be race (white versus black), secondary analyses restricted to blacks alone will be performed using European ancestry as the predictor. Estimates of European ancestry have already been determined as part of a previously approved manuscript proposal using genotyping from the Affymetrix 6.0 array: Locus-specific African ancestry across the genome in African Americans was estimated using the program LAMPLD 1.0 using a 2 population model (African and European). Genotype data for Yoruba in Ibadan, Nigeria and Utah Residents with Northern and Western European Ancestry from The International HapMap Project ([www.hapmap.org](http://www.hapmap.org)) were used as ancestral reference data for the 2 ancestral populations.

After eliminating patients with prevalent disease (i.e. AF or HF for the respective analyses, defined as an AF or HF diagnosis at or before Nt-proBNP measurement), we will examine patients with documented Nt-proBNP levels and basic demographic information, including racial group. This will result over 10,000 participants in from ARIC.

We will initially use logistic regression to assess the unadjusted association between race (and subsequently European ancestry) and each outcome. We will then repeat the analysis after adjusting for age, sex, race, body mass index, smoking status, alcohol consumption, diabetes, hypertension, heart failure (in AF analysis only), coronary artery disease and myocardial infarction, left ventricular hypertrophy, and chronic kidney disease.

Kaplan-Meier estimates of outcome will be compared to cumulative incidence estimates of outcome using death as a competing risk. If the estimates are equivalent in the two models, the simpler Kaplan-Meier models will be used. Cox proportional hazards regression models adjusted for the above covariates will then be used to estimate the race-AF and race-HF associations. Proportional hazard assumptions will be tested using Proportional Hazard Assumption tests (i.e. Log-Log plots of survival, Kaplan-Meier and predicted survival plots, and examining Schoenfeld residuals).

The degree to which the differential race-AF and race-HF associations are mediated by Nt-proBNP will be assessed in the proposed indirect pathway: first, using a linear model for the black/white difference in Nt-proBNP; and second, using a Cox proportional hazards model for the association of Nt-proBNP with incident AF and CHF, adjusting for

race/ethnicity. Assuming both of these analyses are positive, the change in the point estimate between predictor (race or European ancestry) and outcome (AF or CHF) before versus after addition of Nt-proBNP into the multivariate Cox proportional hazards model will then be determined; we will use the “percent treatment explained” approach to derive 95% confidence intervals in assessing this potential mediation. To deal with right-skewness of Nt-proBNP as well as the non-linearity of its association with cardiovascular events, this potential mediator will be log-transformed for analysis. Inputs for our power calculations included the proportions of black participants in ARIC, published incidences of CHF,<sup>29</sup> and AF events in ARIC,<sup>30</sup> an estimate of the standardized black/white difference in log-transformed Nt-proBNP based on data from the Heart and Soul Study [personal communication], and published estimates of the association of log-transformed Nt-proBNP with incident AF.<sup>23</sup> Using recently developed methods,<sup>31</sup> we estimated that the ARIC sample will provide at least 98% power to simultaneously detect both links in the indirect pathway.

Finally, we will repeat the analysis stratified by those with and without baseline hypertension and with and without baseline heart failure; for the HF analysis, we will stratify by baseline hypertension and coronary artery disease.

We plan to repeat these analyses in the Cardiovascular Health Study as validation of our findings.

**7.a. Will the data be used for non-CVD analysis in this manuscript?** \_\_\_\_ 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?** \_\_\_\_ 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 \_\_\_\_ 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.csc.unc.edu/ARIC/search.php>**  
 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)?**

Existing Publications:

- Gupta DK, Claggett B, Wells Q, et al. Racial differences in circulating natriuretic Peptide levels: the atherosclerosis risk in communities study. Journal of the American Heart Association 2015;4.
- Sinner MF, Stepas KA, Moser CB, Krijthe BP, Aspelund T, Sotoodehnia N, Fontes JD, Janssens CAJW, Kronmal RA, Magnani JW et al.. 2014. B-type natriuretic peptide and C-reactive protein in the prediction of atrial fibrillation risk: the CHARGE-AF Consortium of community-based cohort studies.. Europace. 16(10):1426-33.
- Nambi V, Liu X, Chambless LE, de Lemos JA, Virani SS, Agarwal S, Boerwinkle E, Hoogeveen RC, Aguilar D, Astor BC et al.. 2013. Troponin T and N-terminal pro-B-type natriuretic peptide: a biomarker approach to predict heart failure risk--the atherosclerosis risk in communities study.. Clin Chem. 59(12):1802-10.
- Alonso A, Agarwal SK, Soliman EZ, et al. Incidence of atrial fibrillation in whites and African-Americans: the Atherosclerosis Risk in Communities (ARIC) study. American heart journal 2009;158:111-7.
- Marcus GM, Alonso A, Peralta CA, et al. European ancestry as a risk factor for atrial fibrillation in African Americans. Circulation 2010;122:2009-15.

Existing Manuscript Proposals:

The ARIC sponsoring author has not identified any proposals that overlap with this current proposal.

**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.csc.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.csc.unc.edu/aric/index.php>, under Publications, Policies & Forms. [http://publicaccess.nih.gov/submit\\_process\\_journals.htm](http://publicaccess.nih.gov/submit_process_journals.htm) shows you which journals automatically upload articles to Pubmed central.

## 12. References

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