ARIC Manuscript Proposal #3408

PC Reviewed: 6/18/19	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Analysis of Erythropoietin in Genetic Associations and Cardiovascular Traits and Outcomes

b. Abbreviated Title (Length 26 characters): **ARIC EPO Study**

2. Writing Group:

Alanna Morrison
Eric Boerwinkle
Josef Coresh
Bing Yu
Adrienne Tin
Santhi Ganesh

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

First author: Santhi Ganesh
Address: 1150 West Medical Center Drive
MSRB III, Room 7220A
Ann Arbor, MI 48104

Phone: (734)764-4500 Fax: (734)936-8266
E-mail: sganesh@umich.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: Alanna C. Morrison, Ph.D.
Address: University of Texas Health Science Center at Houston
1200 Pressler St., Room E453
Houston, TX 77030
tel: 713 500-9913
fax: 713 500-0900
e-mail: alanna.c.morrison@uth.tmc.edu

3. Timeline:

Completion of analyses and submission of manuscripts by 2020.

4. Rationale:

Cardiovascular disease (CVD) is the leading cause of death globally. Elevated blood pressure (BP), or hypertension (HTN), is a major, heritable risk factor for CVD(Lewington, Clarke et al. 2002). Prior epidemiologic research in large U.S. population cohorts have shown that clinically relevant red blood cell (RBC) traits, such as hemoglobin (Hb) and hematocrit (Hct, the percentage of blood volume occupied by RBCs), increase BP(Havlik, Garrison et al. 1980, Tell, Fried et al. 1993) and are independent risk factors for HTN(Sorlie, Garcia-Palmieri et al. 1981, Carter, McGee et al. 1983, Campbell, Elwood et al. 1985, Erikssen, Thaulow et al. 1993, Gagnon, Zhang et al. 1994, Nishikido, Kobayashi et al. 1999, Skretteberg, Bodegard et al. 2010), ischemic or coronary heart disease (CHD), CVD mortality(Erikssen, Thaulow et al. 1993, Brown, Giles et al. 2001), and all-cause mortality(Elwood, Waters et al. 1974, Zakai, Katz et al. 2005) (HR for RBC traits predicting 1-year death or CHD as high as 4.47 (3.48–5.73 95% CI)(Brennan, Reddy et al. 2010)). The upper and lower tails of the Hct distribution are associated with excess CHD risk. Anemia is present in 3.4 million U.S. adults(Collins, Ma et al. 2000, Sarnak, Tighiouart et al. 2002, Nissenson, Goodnough et al. 2003, Guralnik, Eisenstaedt et al. 2004, Guralnik, Ershler et al. 2005) and is an independent risk factor for CHD. In the Atherosclerosis Risk in Communities (ARIC) Study, the presence of anemia was independently associated with CHD risk (HR1.41, 1.01-1.95, ($p<0.05$) N=14,410)(Sarnak, Tighiouart et al. 2002). Individuals with higher Hct are also at increased risk of HTN, CHD and CHD-related death, and this effect is independent of CVD risk factors, such as body mass index (BMI) or smoking(Sorlie, Garcia-Palmieri et al. 1981, Smith, Lowe et al. 1992), or for plasma volume(Parving and Gyntelberg 1973, Chrysant, Frohlich et al. 1976, Havlik, Garrison et al. 1980, Cohn 1984). Hb or Hct are independently associated with BP as a continuous variable and with HTN(Havlik, Garrison et al. 1980, Garrison, Kannel et al. 1987, Wilking, Belanger et al. 1988) with every 1 point increase in Hb predicted to account for 20 excess cases of HTN in a population such as ARIC (preliminary data).

Erythropoeitin (EPO) is a regulator of red blood cell synthesis in the bone marrow. High blood pressure (BP) is a common adverse effect of exogenously administered erythropoietin (EPO) therapy in patients with chronic kidney disease (CKD) on hemodialysis (Besarab, Bolton et al. 1998, Drueke, Locatelli et al. 2006, Singh, Szczech et al. 2006, Santhanam, d'Uscio et al. 2010, Lundby and Olsen 2011, Agarwal 2017). Other than stimulating the production of new red blood cells (RBC) in CKD patients (Eschbach, Egrie et al. 1987), EPO therapy is associated with increased cardiovascular adverse events and mortality in CKD patients (CHOIR, CREATE studies and NHCT trial) (Besarab, Bolton et al. 1998, Drueke, Locatelli et al. 2006, Singh, Szczech et al. 2006, Santhanam, d'Uscio et al. 2010, Agarwal 2017). The mechanisms contributing to these excess cardiovascular and mortality events on EPO therapy are not fully elucidated, but HTN is considered a leading risk factor (d'Uscio, Smith et al. 2007, Fishbane and Nissenson 2007, Krapf and Hulter 2009). Recent reports suggest that the detrimental effects of EPO may be mediated via its receptors expressed on non-hematopoietic tissues including the vasculature that mediates increase in vascular tone and BP by dysregulating endothelin-1 expression (ET-1), nitric oxide, renin-angiotensin system, prostaglandin

synthesis, and calcium signaling (Watowich 2011, Agarwal 2017). In human genetic studies of individuals in the population, the 7q22.1 EPO locus is associated with not only RBC traits but BP as well (Chang, Liu et al. 2007, Ganesh, Zakai et al. 2009, Levy, Ehret et al. 2009); however cause-and-effect relationships between these genetic variants and the mechanisms of BP alterations remain to be established.

The Ganesh lab is investigating the relationship of EPO to BP and cardiovascular traits in the context of a R01 with several ARIC co-investigators in genetic epidemiology, both at University of Texas in Houston and Johns Hopkins School of Public Health. We are investigating mechanisms of pleiotropy and mediation between genetic loci associated with red blood cell traits, including EPO, and BP, HTN and CVD outcomes. Additionally the Ganesh lab is collaborating with Peking University in China for genetic studies of cardiovascular traits, including EPO directly measured by plasma ELISA, in which known and novel genetic associations have been identified. We are now seeking replication in the ARIC SOMAscan data at visit 5 of these genetic associations as initial step and validation of the SOMAscan data. Once this is completed, we further plan to analyze the association of EPO in ARIC with genome-wide markers and vascular traits including BP (SBP, DBP, MAP, PP), pulse-wave velocity (PWV), and potentially others. We will require several covariates for the analyses as detailed below, relevant to defined conditions in which EPO expression is known to be altered.

The overarching goal of this proposal is to identify novel genetic influences on EPO levels and the impact of EPO on blood pressure, CHD and other cardiovascular traits. We will test the hypotheses that systematic evaluation of genome-wide variants, both common and rare, will uncover genes which play an important role in EPO regulation and downstream cardiovascular impact of EPO levels.

5. Main Hypothesis/Study Questions:

A. What rare and common variants are associated with EPO level in the population?

1. Analyses will be performed using both single variant and gene-based approaches.

2. EPO SOMAscan data at visit 5 will be used, with validation in pooled plasma samples.

B. We will test the hypothesis that EPO levels in the population are associated with blood pressure and other cardiovascular traits as well as CHD outcomes.

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).

We will analyze the following variables: EPO level as determined in the ARIC SOMAscan data at visit 5, blood cell counts, blood pressure, anti-hypertensive medication use, pulse-wave velocity, ankle-brachial index, carotid intima-media thickness and CHD outcomes.

Covariate data: self-reported race, sex, age at measurement, renal disease, smoking status, status of statin use, cancer diagnosis, BMI and lipids (total cholesterol or lipid profile).

Analysis Model:
Genetic associations will be tested using single variant and aggregated variant association tests, using genome sequence data (exomes or genomes) and genome-wide association genotypes that have been imputed to 1000 genomes, Haplotype Reference Consortium and/or TOPMed imputation panels. Linear models for single variants and aggregate tests that focus on particular genomic motifs or sliding windows will be used (Morrison, Huang et al. 2017). Manual/bioinformatic annotation of key genes including EPO and the EPO receptor will be done as well. Additional traits will be analyzed in logistic or linear regressions.

Analysis Plan:
Our primary analyses will focus on 4 main quantitative traits: EPO, hemoglobin, hematocrit, and blood pressure (SBP, DBP, MAP and PP). Additional traits will include red cell indices and other blood cell counts, renal function, and dichotomous traits and outcomes including HTN and CHD. Anticipated exclusion criteria are congenital anemia, or concurrent diagnosis of hematologic cancer or receiving myelosuppressive chemotherapy. End-stage renal disease may be considered in a subset analysis representing a high-risk cohort for EPO-associated outcomes. The use of exogenous EPO therapy, in the setting of renal failure or cancer, will be examined.

We will perform a number of preliminary QC steps, including plotting the distribution of betas and standard errors by study and plotting the allele frequencies of variants compared to the weighted average of the allele frequencies across all studies. QQ-plots will be examined at different points in the analysis in order to identify potential issues that would require additional refining of sample and variant filters.

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)?**

There are no manuscript proposals for the evaluation of EPO associations or this use of the soma scan data, confirmed with Dr. Josef Coresh. Our collaborative group is already studying interactions of hematology traits, blood pressure and other cardiovascular traits and outcomes.

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? _X___ Yes ___ No

This proposal is related to the ancillary study proposal 2012.05 Exome and whole genome sequence analysis of blood cell traits

11.b. If yes, is the proposal

___ A. primarily the result of an ancillary study (list number* 2012.05)

___ 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.

13. References

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