ARIC Manuscript Proposal #2659

PC Reviewed: 11/10/15	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Relationship between common and rare variants in the *SGLT2* gene and Blood Pressure, Cardiovascular outcomes, mortality and Congestive Heart Failure in the ARIC study

b. Abbreviated Title (Length 26 characters): *SGLT2* genetics and CVD outcomes

2. Writing Group:

Writing group members: Sara B. Seidelmann, Brian Claggett, Linda Polfus, Susan Cheng, Eric Boerwinkle, Calum MacRae, [OTHERS WELCOME], Scott D. Solomon

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

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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).

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3. Timeline: Analysis will begin following proposal approval with the aim of completing the analysis and associated manuscript(s) within 6 months to 1 year of data availability.

4. Rationale:

The kidney has been increasingly recognized as an important regulator of glucose homeostasis historically through gluconeogenesis but more recently through glucose excretion. The pharmacological inhibition of renal glucose reabsorption is a novel approach to the treatment of type-2-diabetes (T2DM). Sodium-glucose co-transporter 2 (SGLT2) inhibitors are a relatively new class of FDA approved drugs that inhibit renal proximal tubular glucose reabsorption with the additional benefits of decreases in body weight and blood pressure with a low risk of hypoglycemia due to their insulin-independent mechanism of action. The recently published EMPA-REG trial showed that an SGLT2 inhibitor reduced mortality and hospitalization for heart failure[1].

SGLT2 is encoded by the *SGLT2* gene (OMIM 182381) on chromosome 16 and catalyzes the active transport of glucose against a concentration gradient across the luminal membrane by coupling it with the transport of sodium. Genetic variations in the *SGLT2* gene have long been proposed to contribute to renal glucosuria (OMIM 233100) in subjects [2, 3]. Except for increased renal excretion of glucose, individuals with familial renal glucosuria are largely asymptomatic. Two types of familial renal glucosuria have been described—Type A in which there is reduced expression of the SGLT2 protein, and Type B in which the SGLT2 protein has a reduced affinity for glucose. In both types, affected subjects have a reduced glucose reabsorption threshold, but are generally healthy with normal blood glucose levels although long-term studies are extremely limited. Therefore, renal glucosuria has been deemed a "nondisease" defined by urinary glucose excretion in the presence of normal blood glucose concentrations and in the absence of general renal tubular dysfunction. The prevalence of glucosuria in the population is estimated at 0.16-6.3%. Inheritance patterns of renal glucosuria most commonly show codominance with variable penetrance [4], although autosomal recessive patterns have been reported [5, 6].

More recently, one small study looked at the contribution of common genetic variation in *SGLT2* on glucose traits in non-diabetic subjects. The rs9934336 G-allele was associated with increased 30-min plasma glucose, 120-min insulin concentrations and AUC120minglucose during oral glucose tolerance test in 907 non-diabetic individuals [7]. In the combined analysis including another independent cohort, rs9934336 was associated with 120-min insulin concentrations in nondiabetic subjects (n = 2590). This suggests that common variants in *SGLT2* could also play a role in regulation of glucose and insulin levels.

The primary benefit of the SGLT2 inhibitor in the EMPA-REG study appears to be reduction in heart failure hospitalization and related mortality, possibly through a direct diuretic effect. We thus hypothesize that variation in this gene may play a role in sodium and water homeostasis, and may effect blood pressure, or modify the influence of diabetes and blood pressure on incident heart failure.

5. Main Hypothesis/Study Questions:

1. We hypothesize that the presence of mutations in *SGLT2* may be associated with markers of glucose, insulin, sodium and water homeostasis and with cardiovascular outcomes, including heart failure.
2. We hypothesize that rare, including loss of function variants in *SGLT2* will influence cardiovascular outcomes and overall mortality.
3. We hypothesize that common variants in *SGLT2* may also be associated with the above phenotypic measures in subjects in the ARIC cohort.

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).

Exposures:
Common variants in and around the SGLT2 gene (n=13,087 on Affymetrix 6.0 array) will be analyzed. Available whole exome/genome sequencing and Exome chip data obtained from the study participants (N=14,106 on Illumina Human Exome Bead Chip, version 1.0) will be analyzed to identify subjects with rare *SGLT2* gene variants (MAF <1%). Variants will be annotated to determine whether they are likely benign versus pathogenic. Nonsense, frame shift, and splice site variants will be considered to be pathogenic. Missense variants will be considered pathogenic based on concordant SIFT (<0.02) and PolyPhen-2 scores (>0.90).
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Outcomes:
Clinical events--The outcomes studied will be cardiovascular events (including death) since the first visit. New stroke (fatal or non-fatal) or incident CHD (fatal or non-fatal MI or CHD death) since the first visit to the fifth visits (2011-2013) among subjects who were free of these outcomes at the beginning of visit one. CHF, including both systolic heart failure and heart failure with preserved ejection fraction will also be evaluated. HF treatment prior to event (ACEi, ARB, beta blocker, MRA, digoxin, statins, diuretics) will also be evaluated.

Blood Pressure - systolic and diastolic blood pressure measured at visits 1-5 and hypertension status will be evaluated.

Diabetic and metabolic related phenotypes-- Total cholesterol, high density lipoprotein, diabetes mellitus status, HbA1C, body weight, BMI, waist-to-hip ratio, percent body fat, fat mass, lean body mass, fasting glucose, insulin levels will be assessed. Oral glucose tolerance test results will also be assessed.

Covariates:
Age, gender, race.

Analysis plan:

The genetic analysis will be stratified by race.
Descriptive statistics: Patient characteristics will be compared between those with rare pathogenic variants in *SGLT2* and those without. Categorical data will be displayed as percent frequencies and compared by $\chi 2$ or Fisher exact tests. Continuous data will be displayed as means (±SD) for normally distributed variables and medians (interquartile range) for variables with skewed distributions. We will utilize regression models for the analysis, designating the phenotype as dependent variable and rare variants as independent or predictor variables. We will utilize regression models for the analysis, designating the phenotype as dependent variable and rare variants (using T1 and SKAT) as independent or predictor variables. For quantitative phenotypes, we will create basic as well as age and gender adjusted models to test associations. We will also compare right and left ventricular continuous and categorical echocardiographic parameters, and quantitative diabetic and metabolic traits between rare variant carriers and non-carriers as above. Basic as well as age- and gender-adjusted hazard ratios will be used to test the association between pathogenic rare variant carriers and the risk of death, cardiovascular outcomes and heart failure using Cox proportional hazards regression. Common *SGLT2* variants and their association with quantitative and categorical variables will be tested using regression modeling (crude and age/sex adjusted) assuming an additive and/or recessive model. Risk of death, cardiovascular outcomes and heart failure will be assessed using Cox proportional hazards regression.

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)?

ARIC Manuscript Proposal #2068. Exome chip and exome sequencing analyses for BP phenotypes. Corresponding author Eric Boerwinkle.

Past (inactive) proposals

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.

13. Per Data Use Agreement Addendum for the Use of Linked ARIC CMS Data, approved manuscripts using linked ARIC CMS data shall be submitted by the Coordinating Center to CMS for informational purposes prior to publication. Approved manuscripts should be sent to Pingping Wu at CC, at pingping_wu@unc.edu. I will be using CMS data in my manuscript ____ Yes __x__ No.

References:

1. Zinman, B., et al., *Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes.* N Engl J Med, 2015.
2. van den Heuvel, L.P., et al., *Autosomal recessive renal glucosuria attributable to a mutation in the sodium glucose cotransporter (SGLT2).* Hum Genet, 2002. **111**(6): p. 544-7.
3. Kanai, Y., et al., *The human kidney low affinity Na+/glucose cotransporter SGLT2. Delineation of the major renal reabsorptive mechanism for D-glucose.* J Clin Invest, 1994. **93**(1): p. 397-404.
4. Santer, R., et al., *Molecular analysis of the SGLT2 gene in patients with renal glucosuria.* J Am Soc Nephrol, 2003. **14**(11): p. 2873-82.
5. Elsas, L.J., D. Busse, and L.E. Rosenberg, *Autosomal recessive inheritance of renal glycosuria.* Metabolism, 1971. **20**(10): p. 968-75.
6. Khachadurian, A.K. and L.A. Khachadurian, *The Inheritance of Renal Glycosuria.* Am J Hum Genet, 1964. **16**: p. 189-94.
7. Enigk, U., et al., *Role of genetic variation in the human sodium-glucose cotransporter 2 gene (SGLT2) in glucose homeostasis.* Pharmacogenomics, 2011. **12**(8): p. 1119-26.