ARIC Manuscript Proposal #3596

PC Reviewed: 4/14/20	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Urine Biomarkers Associated with Incident Chronic Kidney Disease in Three Studies of Individuals With Preserved Kidney Function and Without Diabetes: ARIC, REGARDS, and MESA

b. Abbreviated Title (Length 26 characters): Urine Biomarkers and CKD

2. Writing Group:

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On behalf of the Chronic Kidney Disease Biomarkers Consortium

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. _CMR____ **[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: The timeline is approximately six months. Data analysis will begin immediately upon approval of the manuscript proposal. It is anticipated that a manuscript draft will be shared with the writing group in June 2020, submitted to the ARIC Publications Committee and other relevant publication committees (MESA, REGARDS, Chronic Kidney Disease Biomarkers Consortium) in July 2020, and submitted to a journal in August 2020.

4. Rationale:

Chronic kidney disease (CKD) is a major public health issue that is an independent risk factor for cardiovascular disease and death. Available treatments to arrest CKD progression are limited and even with optimal treatment, outcomes in CKD remain poor (1). Of those individuals who have CKD stage 3, 11% will eventually require renal replacement therapy for ESRD, resulting in considerable cardiovascular disease and other morbidity, as well as significant costs, currently estimated at $23 billion annually. Few therapies are effective at slowing the progression of CKD (2). Renin-angiotensin-aldosterone system blockers can delay progression of CKD in individuals with diabetes in particular, but have limited efficacy in non-proteinuric CKD (3-5). Thus, there is a need for new therapies to further delay or stop the progression of CKD.

One of the major limiting factors in the development of novel therapies is the lack of sensitive, early biomarkers of acute and chronic kidney injury. Serum creatinine, the current primary biomarker of CKD has important limitations. These include variation with race and muscle mass which are independent of GFR along with significant extra-renal clearance. However, the most important limitations are that it is a relatively insensitive marker of early kidney damage and it provides no information as to the site of renal injury.

Thus, the future development of therapies that can prevent progression of CKD depends on the ability to identify individuals at risk of progression prior to any rise in the serum creatinine while also accurately localizing the site of injury without the need for kidney biopsy. If validated, these biomarkers could serve as surrogate markers of kidney injury for future clinical trials and in clinical practice. The urine biomarkers to be examined in this particular proposal are: EGF, MCP-1, KIM-1, YKL, α1-microglobulin. These biomarkers were measured at the Bonventre laboratory at Brigham and Women's Hospital.

Urine kidney injury molecule-1 (KIM-1) is a protein expressed by kidney tubule cells in response to injury and shed into the urine. Urine monocyte chemoattractant protein-1 (MCP-1) is a protein expressed in response to tubule cell injury as a method to attract inflammatory cells, particularly monocytes, in response to injury. Urine epidermal growth factor (EGF) differs from the markers above, as lower levels, rather than higher levels, are hypothesized to be associated with CKD progression. Deletion of the EGF receptor in proximal tubule cells delays regeneration

of tubule epithelium in mice subjected to renal ischemia-reperfusion injury, whereas administration of EGF reduced apoptosis in an experimental model of kidney fibrosis. In humans, lower urine EGF was associated with slower recovery from AKI, and persons with IgA nephropathy have lower urine EGF excretion. Chitinase-3-like protein-1 (aka YKL-40) functions as a mediator of the reparative response to tubule injury. Finally, urine alpha 1 microglobulin (α1M) is a small protein that is freely filtered at the glomerulus, but then avidly reabsorbed at the proximal tubule such that many healthy individuals do not have detectable α1M in the urine. Higher levels of α1M mark diminished proximal tubule reabsorptive capacity.

5. Main Hypothesis/Study Questions:

To examine the prospective associations of novel urine biomarkers with risk of incident chronic kidney disease among individuals without diabetes and with preserved kidney function at baseline.

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: This ARIC case-cohort study includes 446 incident CKD cases and a sub-cohort of 428 individuals. Ascertained at ARIC visit 5, we defined the incident CKD cases as non-diabetic and with eGFR>60 mL/min/1.73 m^2 at visit 4 and have eGFR declined to less than 60 mL/min/1.73 m^2 and 40% or more decrease at visit 5. A similar study design has been used for MESA and REGARDS.

Inclusion/Exclusion Criteria: We included all eligible cases ascertained at ARIC visit 5 (ascertainment of the outcome for case definitions occurred at visit 5; visit 4 specimens were used for assays) and a randomly selected sub-cohort. We excluded individuals who had missing eGFR at visit 4 or visit 5 or eGFR less than 60 mL/min/1.73 m^2 at visit 4. We also excluded individuals who had a missing value for baseline covariates. For this study, participants with diabetes at baseline (visit 4) were excluded.

Outcome: The primary outcome is eGFR decrease by >40% to a level <60 ml/min/1.73 m^2 or ESRD. Given that the ascertainment of the primary outcome occurred at visit 5, the time to onset of incident CKD will not be known.

Statistical Analysis: Urinary biomarkers will be indexed to urinary creatinine to account for variability in fluid excretion. We will also conduct an analysis without indexing to urine creatinine, but will include urine creatinine (as well as urine albumin) as a covariate. We will assess baseline characteristics in the overall study population and according to quartiles of each biomarker. We will assess the cross-sectional correlation between all of the urine biomarkers, and with UACR and eGFR.

Next, to explore the relationships of each biomarker with incident CKD, we will use multivariable logistic regression regression models. We will conduct continuous analyses of the biomarkers, expressed per doubling of the biomarker level. We will also explore associations of

biomarker quartiles, setting the lowest quartile as the reference category, in order to explore the functional form of the relationships. We will conduct a sequence of models. An initial model will be unadjusted. A second model will adjust for demographics (age, sex, race, education level) and urine creatinine concentration. A third model will additionally include CKD risk factors (BMI, SBP, BP med use, smoking status). Finally, the fourth model will include eGFR and urine albumin concentration.

We will also explore analyses that assess the association between the panel of urine biomarkers and incident CKD. For example, we will include all the biomarkers in the same model.

The results for each study (ARIC, MESA, REGARDS) will be presented together in a single manuscript. We will conduct a meta-analysis of summary estimates for each biomarker using random effects models. Heterogeneity will be assessed using Q and I^2 statistics.

Anticipated Methodologic Challenges: The main challenge is the anticipated differences in results across studies. We will report tests of heterogeneity as a quantitative measure to assess these differences. We will also explore study-specific associations between biomarkers and baseline characteristics and quantify correlations between biomarkers and kidney measures (UACR, eGFR). All of these results will be discussed as potential explanations for differences in the results across the three studies.

7.a. Will the data be used for non-ARIC analysis or by a for-profit organization in this manuscript? ____ Yes __X__ No

b. If Yes, is the author aware that the current derived consent file ICTDER05 must be used to exclude persons with a value RES_OTH and/or RES_DNA = "ARIC only" and/or "Not for Profit" ? ____ Yes ____ No
(The 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 current derived consent file ICTDER05 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/mantrack/maintain/search/dtSearch.html

___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)?

2177 – Association of urinary biomarkers with the risk of end-stage renal disease in the general population: CKD Biomarkers Consortium (First author: Meredith Foster)

This existing manuscript proposal also studies urine biomarkers, including KIM-1, but was conducted in a different sub-sample within ARIC. In this second phase of CKD Biomarkers Consortium, we have assayed multiple novel biomarkers that have not been previously studied in the ARIC study.

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* 2017.13**)**

___ 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 https://sites.cscc.unc.edu/aric/approved-ancillary-studies

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.