

ARIC Manuscript Proposal # 1544

PC Reviewed: 8/11/09
SC Reviewed: _____

Status: A
Status: _____

Priority: 2
Priority: _____

1.a. **Full Title:** Urinary proteins and incident chronic kidney disease

b. **Abbreviated Title (Length 26 characters):** Urinary biomarkers of CKD

2. **Writing Group:**

Writing group members: Brad Astor, Anna Kottgen, Joe Coresh, others welcome

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. BA **[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:** We expect that the laboratory assays will be completed within 3 weeks. We hope to complete analyses and manuscript development quickly, in order to follow-up on the recently published GWAS discovery. We expect the manuscript to be completed within 3 months.

4. **Rationale:**

We recently published results from a genome-wide association study which identified 7 SNPs in or upstream of the *UMOD* (uromodulin) gene as associated with chronic kidney disease.¹ Rare *UMOD* mutations cause autosomal dominant forms of kidney disease. Following-up on this important discovery may lead to new insights about kidney disease pathogenesis.

Tamm-Horsfall Protein (THP, also known as uromodulin), the product of *UMOD*, is the most abundant protein in human urine, and is produced only in the thick ascending limb of the loop of Henle.² Its function has not been fully characterized, but studies have demonstrated that THP plays a role in clearing bacteria, thereby protecting the urinary tract from infection, and in precluding urinary crystal formation. As THP has been shown to bind to a variety of cells, ions, immunoglobulins and cytokines, it is hypothesized that THP may play a role in the binding and excretion of products potentially injurious to the kidney.^{3;4}

Measurement of THP is performed on a multiplex panel, which includes additional analytes, including two markers of acute kidney injury, neutrophil gelatinase-associated

lipocalin (NGAL) and Kidney Injury Molecule-1 (KIM-1). Small studies suggest that these markers are correlated with kidney function, and also may predict progression of chronic kidney disease.^{5,6}

We will analyze urinary levels of THP, NGAL, KIM-1, and other urinary markers listed below in Visit 4 urine samples from cases and non-cases of incident chronic kidney disease (CKD) occurring between ARIC Visit 4 and ARIC Carotid MRI.

Depending on the results of analyses, we may choose to split the proposed manuscript into separate manuscripts for each of the relevant urinary biomarkers.

5. Main Hypothesis/Study Questions:

Hypothesis: Urinary THP levels are higher in cases of incident CKD (defined below) than non-cases of incident CKD.

6. Design and analysis

Design: Case-control – Cases of incident CKD, defined by the change in estimated glomerular filtration rate (eGFR) from ARIC Visit 4 (baseline) to ARIC Carotid MRI (follow-up). Cases will be defined as having a baseline (Visit 4) eGFR above 60 ml/min/1.73m² and a follow-up (Carotid MRI) eGFR below 60 ml/min/1.73m² AND a drop in estimated GFR of at least 25%.

Controls will be frequency matched on age (5 year categories), sex, race and presence of albuminuria (< or ≥ 30 mg/g albumin to creatinine ratio).

Approximately 168 cases and an equal number of controls will be included in the analyses.

Inclusion criteria: Serum creatinine and urinary albumin and creatinine measured at Visit 4. Serum creatinine measured at Carotid MRI. Available urine sample from Visit 4.

Predictors:

Tamm-Horsfall protein (THP)

We recently completed a pilot study of 42 samples evenly divided by genotype, which showed that genotype levels are associated with 50% variation in THP in urine (p-trend=0.01). We confirmed the validity of the immune-assay used by RBM by comparing the results to THP quantification done by silver stained gels (correlation 0.8 including 2 outliers; gel precision is lower in general). We are coordinating with Framingham (PI:Fox) a joint publication on this topic. These are the first data on this topic of which we are aware.

The proposed assay has a lower limit of detection of 0.46 ug/mL (dynamic range 1.6-800) and inter-assay CV of 5-11% at the three levels tested. Stability studies suggest some loss when stored for 4 hours at room temperature (88% recovery in urine vs. 99% at 2 hours). While the levels in some normal individuals will be at the lower limit, the assay performance characteristics are promising.

In our pilot data we assayed all samples in duplicate and found a correlation of 0.996 between duplicates suggesting single assays (as suggested by RBM) are adequate.

Neutrophil gelatinase-associated lipocalin (NGAL)

NGAL, a protein in the lipocalin family, has been studied extensively in acute kidney injury and is strongly expressed following ischemic or nephrotoxic injury in animals and humans.⁷⁻⁹ Recent studies of NGAL have shown that the protein may also predict chronic kidney disease.^{10;11} In one study of 45 patients with CKD secondary to renal dysplasia, obstructive uropathy, and glomerular and cystic diseases, plasma NGAL levels were inversely associated with glomerular filtration rate (GFR).¹¹ In a separate study conducted in 33 CKD patients and 20 controls, mean urinary NGAL levels were higher in CKD individuals (378.28 ± 111.13 ng/mL compared to 7.38 ± 3.26 ng/mL; $p=0.01$).¹⁰ Additionally, urinary NGAL levels were significantly correlated with serum creatinine ($r=0.588$; $p=0.02$), GFR ($r=-0.528$; $p=0.04$), and proteinuria ($r=0.294$; $p=0.01$).

The proposed assay has a lower limit of detection of 4.1 ng/mL (dynamic range 1.2-6000) and inter-assay CVs of 5%-13% across 3 levels tested. Stability studies suggest good stability up to 24 hours at 4C. While the levels in some normal individuals will be at the lower limit, the assay performance characteristics are promising.

Kidney Injury Molecule-1 (KIM-1)

Kidney Injury Molecule-1 is a transmembrane tubular protein that is undetectable in normal kidneys but shows expression following injury to the kidney.¹² Animal models have shown that expression of KIM-1, a type 1 transmembrane protein, is potently up regulated in the proximal tubule region of the kidney post-ischemic injury.¹³ Human studies have found that urinary KIM-1 is associated with inflammation and renal function and suggest that it may be used as a non-invasive biomarker of renal dysfunction.¹³ Data on the association between KIM-1 and risk of incident kidney disease are limited.

The proposed assay has a lower limit of detection of 0.028 ng/mL (dynamic range 0.005-24) and inter-assay CVs of 4%-13% across 3 levels tested. Stability studies suggest good stability for 2 hours at 4C for 16% degradation after 4 hours at room temperature suggesting timely handling of specimen is warranted. While the levels in some normal individuals will be at the lower limit, the assay performance characteristics are promising.

Additional Analytes: Rules Based Medicine's Human KidneyMAP™ panels include 14 analytes:

Alpha-1 Microglobulin
Beta-2 Microglobulin
Calbindin
Clusterin
Connective Tissue Growth Factor (CTGF)
Cystatin C
Glutathione S-Transferase alpha (GST alpha)
Kidney Injury Molecule-1 (KIM-1)
Neutrophil Gelatinase-Associated Lipocalin (NGAL)
Osteopontin
Tamm-Horsfall Protein (THP)
Tissue Inhibitor of Metalloproteinase-1 (TIMP-1)
Trefoil Factor 3 (TFF3)
Vascular Endothelial Growth Factor (VEGF)

Many of these analytes are of substantial interest. Since the three analytes of interest THP, NGAL and KIM-1 are on 3 separate multi-plexes, which include a total of 14 analytes, we opted to run the entire panel, maintaining its integrity and multiplex optimization.

Data analysis: Conditional logistic regression will be used to account for the frequency matching. The weighted design of the MRI study and its implication for analyses of case-control studies will be discussed with the coordinating center. Preliminary discussion suggests two alternative approaches to account for the stratified sampling: 1) using the stratification variables for our selection of controls and the stratification variables introduced by sampling for ARIC Carotid MRI (center, high/low IMT by ultrasound) and making inferences to the ARIC population or, 2) using only our stratification variables and making inferences to the population sampled for ARIC Carotid MRI, acknowledging that this population over-represents individuals with thick carotid. Review of the initial sample and matched controls shows the expected results. Matching provides good equality across age, sex, and race. Hypertension and diabetes have a higher frequency among cases.

7.a. Will the data be used for non-CVD analysis in this manuscript? ☒ **X** **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? ☒ **X** **Yes**
☐ **No**

(This file ICTDER03 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.csc.unc.edu/ARIC/search.php>

☒ Yes ☐ No

No overlap found with existing manuscript proposals.

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)?

None identified

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

11.b. If yes, is the proposal

☒ **A. primarily the result of an ancillary study (list number* _2006.16_)**
☐ **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.csc.unc.edu/aric/forms/>

12. 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.

Agreed

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