1.a. Full Title: Variants in 10 kidney disease and 272 cardiovascular disease candidate genes and renal phenotypes in the ARIC-MRI Study

b. Abbreviated Title (Length 26 characters): CVD candidate genes and nephropathy

2. Writing Group:

Writing group members: A. Kottgen, L. Kao, E. Boerwinkle, J. Coresh, LE Chambless, others welcome

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

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3. Timeline: Analyses to start immediately after approval, completion of analyses and writing of a manuscript projected within 6 months.
4. Rationale:

Chronic kidney disease (CKD) is a growing epidemic in the United States.\(^1\) Increased excretion of albumin with the urine, albuminuria, is one of the earliest signs of CKD and, along with the estimated glomerular filtration rate (eGFR), can be used to diagnose and stage CKD.\(^2\) The prevalence of elevated levels of albuminuria and of reduced levels of eGFR (\(<60 \text{ ml/min/1.73m}^2\)) in U.S. adults in 2000 has been estimated as 10% and 8%, respectively,\(^1\) and is higher among those with the cardiovascular risk factors diabetes and hypertension. Multiple studies have confirmed that individuals with elevated levels of albuminuria are at increased risk of cardiovascular morbidity and mortality as well as progression to end-stage renal disease,\(^3-6\) as is observed for individuals with reduced levels of eGFR.\(^7,8\) Moreover, the pathogenesis of CKD appears to be similar to the atherosclerotic process, and both share many risk factors such as hypertension. Therefore, CKD and cardiovascular disease may also share common susceptibility genes, and evidence from the ARIC Study supports this hypothesis.\(^9,10\)

Measures of renal damage and function, such as albuminuria and eGFR, have heritable components.\(^11,12\) Although many genetic mutations have been identified that cause monogenetic forms of kidney disease, these variants are usually very rare and do not account for a significant proportion of kidney disease on the population level. Data from recent genome-wide association studies show that common genetic variants increase the susceptibility to complex diseases in the population.\(^13,14\) Some of the variants implicated as increasing susceptibility to complex forms of the disease are located in the same genes that harbor rare mutations causing familial syndromes of the same or a very similar phenotype.\(^15\) We therefore propose to study the association between variants in 272 candidate genes for cardiovascular disease as well as variants in 10 candidate genes specifically for kidney disease with albuminuria and eGFR in the ARIC-MRI Study. Cardiovascular disease candidate genes for inclusion into the ARIC-MRI panel were selected by ARIC investigators based on literature review and their own previous data. Candidate genes specific to kidney disease include genes i) in which rare mutations cause familial forms of kidney disease (\(BSND,^{16} CLCNKB,^{17} SLC12A1,^{18} SLC12A3,^{19}\) and \(TRPC6^{20}\)) ii) that have been associated with kidney disease in the previous literature (\(ACE,^{21} ELM01,^{22} RAGE^{22}\)), and iii) that have been implicated in our prior, not yet replicated or published, association studies of common polymorphisms and kidney disease (\(CD40, KIF6\)).

5. Main Hypothesis/Study Questions:

Main hypothesis: Common genetic variants in renal or cardiovascular disease candidate genes are associated with increased albuinuria and reduced eGFR in ARIC-MRI Study participants.

Study questions:

1. Will there be an association of the genetic variants cross-sectionally with levels of albuminuria and eGFR?
2. Will such an association be consistently observed for both increased albuminuria and reduced eGFR as different renal phenotypes potentially providing insight into the pathophysiologic mechanisms involved?
3. How will such associations be influenced by adjustment for or stratification on other risk factors for kidney disease such as diabetes mellitus and hypertension?
4. Will the association of such genetic variants and renal phenotypes be present in both African American and Caucasian individuals?

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: The ARIC-MRI Study is a substudy among 2066 selected participants of the ARIC Cohort. Participants in this substudy were examined at a study visit in 2005-06.

Inclusions/exclusions: Participants who did not consent to genotyping will be excluded from analysis. Individuals missing variables needed to calculate eGFR and urine albumin-to-creatinine ratio as a measure of albuminuria will be excluded. Moreover, exclusions will be made of individuals or genetic variants that do not meet genotyping quality control criteria. Further exclusion may be made for analyses of different subgroups.

Outcome: The primary outcome will be cross-sectional measures of ACR (n=2007, 480 African American and 1527 Caucasian) and eGFR (n=2024, 491 African American and 1533 Caucasian) at the ARIC-MRI visit. Most analyses will be conducted with a natural logarithmic transformation of these variables partly because of the skewed nature and also to avoid undue influence of extremely high values when the hypothesized genetic effect should be of moderate size. In secondary analyses, ACR and eGFR measured at ARIC cohort visit 4 will be considered to increase the signal to noise ratio (average ACR and eGFR as well as random effects models for repeated measures will be considered). In addition, a time-weighted average measure of creatinine across all 5 ARIC Study visits will be used to calculate eGFR. The urinary albumin-to-creatinine ratio (ACR, mg/g) will be calculated from measurements of urinary creatinine and albumin. Estimated GFR will be calculated from standardized serum creatinine values using the abbreviated Modification of Diet in Renal Disease (MDRD) Study formula: eGFR (ml/min/1.73m² = 175 * (standard creatinine)⁻¹.⁰¹⁵⁴ * age⁻⁰.²⁰₃ * (0.⁷₄₂ if female) * (1.₂₁ if black).²³ The ARIC MRI study used the Roche enzymatic assay for serum creatinine. This assay was calibrated by the manufacturer to be traceable to reference methods (standard creatinine). When compared to the same assay conducted on a calibration panel of 40 samples in the Cleveland Clinic (where the assay was confirmed to yield an unbiased estimate of standard creatinine), the ARIC laboratory showed no meaningful bias in the 0.5 to 2.0 mg/dl range (bias of 0.⁰² mg/dl at a creatinine of 1.⁰ mg/dl) and is therefore used as a measure of standard creatinine in GFR estimation.
**Other variables of interest:** Age, sex, study center, race, CKD risk factors (hypertension, diabetes mellitus, obesity, blood lipids, smoking, prevalent coronary heart disease). Treatment with ACE inhibitors and ARBs will be considered in analyses of ACR as they reduce albuminuria. Analytical approaches to address this issue include adjustment for intake of these medications, use of the ACR and eGFR measurement from cohort visit 4 if treatment was initiated after cohort visit 4, and exclusion of individuals taking these medications. All variables will be used from the ARIC-MRI visits, except for secondary analyses, where measurements of ACR and eGFR from ARIC cohort visit 4 may be used.

**Data analysis:**

*Data checks:* All analyses will be race-stratified. Call rates for all single nucleotide polymorphisms (SNPs) will be determined across and within individuals. Allelic and genotypic frequencies will estimated by race and used to test for departures from Hardy-Weinberg equilibrium. Patterns of linkage disequilibrium will be determined. Differences in the distribution of genotypes between individuals included in the study and those excluded from our analyses will be conducted.

*Data analyses:* In the analyses, sampling scheme into the ARIC Carotid MRI substudy will be accounted for. Associations between each SNP and cross-sectional renal phenotypes will be determined using separate multiple linear regression analyses, adjusting for demographic and kidney disease risk factors. Multivariable adjusted logistic regression analyses will be used to determine the odds of increased albuminuria (ACR >30 mg/g, n=310, 100 African American and 210 Caucasian) and reduced eGFR (<60 ml/min/1.73m², n=357 cases, 72 African American and 285 Caucasian) by genotype status. Additional analyses will also be performed stratified on the CKD major risk factors diabetes and hypertension. In all regression analyses, an additive genetic model will be assumed unless indicated otherwise by results of the analysis, or unless the allele frequency of a given candidate variant is low, in which case a dominant model combining the risk of heterozygotes and homozygotes for the rare allele will be used. Haplotype analyses may be performed, specifically for the 10 kidney disease candidate genes. Haplotype structure will first be evaluated using the program Haploview, and associations between each haplotype and renal phenotypes will be evaluated using the generalized linear model (GLM) method (Haplo.glm). Differences in associations between African Americans and Caucasians will be determined using a Mantel-Hanzel test.

Multiple testing issues will be addressed by evaluating consistency between the two racial/ethnic groups, as well as by attempting to replicate results in other study samples. Replication of results in one or more larger, independent study sample/s, together with a thorough literature review, will be used to separate true from false positive findings, rather than statistically correcting for the number of tests conducted. Statistical correction for the number of test carried out may be conducted for the 10 candidate genes for kidney disease, as these have been implicated in kidney disease development or progression previously. However, replication in an independent study sample, which includes the participants of the ARIC cohort that were not part of the ARIC-MRI Study, remains the preferred method of verification of findings among this
subset of genes as well. Criteria for a genetic variant to be carried forward for replication in a second sample will be based on application of the false positive report probability (FPRP)\(^2\) to all SNPs that show association with the primary outcome in either race at an \(\alpha\)-level of 0.05. The prior probability \(\pi\) of a true association of the tested genetic variant with the outcome, that must be specified to apply the Bayesian FPRP approach, will be set at \(\pi = 0.1\) (high probability) for the CKD candidate genes and \(\pi = 0.001\) (low probability) for the cardiovascular disease candidate genes, with individual SNPs most likely having a somewhat higher or lower prior. A FPRP level of “noteworthiness” will be chosen at 0.5, i.e. the probability of a true association between the genetic variant and the primary outcome given a statistically significant finding will be more likely than not for values below 0.5, and these genetic variants will be carried forward into the replication stage.

The publications committee will be notified if the paper will include a replication dataset from Framingham or another study.

**Limitations:** Cross-sectional measures of kidney function, possibility of survival bias impacting the results, since ARIC-MRI participants were selected among survivors. There will be limited power to investigate genetic variants with low minor allele frequencies.

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 ICTDER02 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 ICTDER02 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 ICTDER02 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/search.php](http://www.cscc.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)?

**MP 984:** Genetic Risk Factors for Nephropathy in the ARIC Study: GLUT1, ZO-1 and NPHS2

**MP 1203:** Association between candidate genetic variants and incident chronic kidney disease: The Atherosclerosis Risk in Communities (ARIC) Study

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/](http://www.cscc.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.

References


