ARIC Manuscript Proposal #3169 (amended)

PC Reviewed: 12/10/19	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: *APOL1* risk variants in a community-based older population

b. Abbreviated Title (Length 26 characters): *APOL1* and older adults

2. Writing Group:
Writing group members: Teresa K. Chen, Morgan E. Grams, Josef Coresh, Adrienne Tin, Shoshana H. Ballew, *others welcome* (order TBD).

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

First author: Teresa K. Chen
Address: 301 Mason F. Lord Drive, Suite 2500
Baltimore, Maryland 21224
Phone: 410-550-2820 Fax: 410-550-7950
E-mail: tchen39@jhmi.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: Morgan E. Grams
Address: 2024 East Monument Street, Suite 2-600
Baltimore, Maryland 21287
Phone: 410-287-1827 Fax: 410-955-0485
E-mail: mgrams2@jhmi.edu

3. Timeline: Analyses will begin once the manuscript proposal has been approved. We anticipate that the manuscript will be written and submitted to the ARIC Publications Committee within one year of the manuscript proposal being approved.

4. Rationale: The *APOL1* high-risk genotypes, present in 13% of black Americans, have been associated with an increased risk for various types of kidney disease, including focal segmental glomerulosclerosis, HIV-associated nephropathy, and hypertension-attributed chronic kidney disease (CKD).[1-4] In the general population, these risk variants have also been associated with kidney disease.[5,6] Utilizing data from the ARIC study, Foster *et al.* reported that individuals with two copies of the *APOL1* risk variants (G1/G1, G1/G2, or G2/G2) had a higher risk of developing incident CKD and end-stage renal disease (ESRD) compared to individuals with one

or no risk variants.[6] Grams *et al.* expanded upon these findings by extending follow-up to Visit 5 and considering additional outcomes. They reported that the *APOL1* high-risk genotypes were associated with increased risk of incident ESRD but not incident hospitalizations or all-cause mortality. While the *APOL1* high-risk genotypes were associated with faster eGFR decline, there was significant overlap in rate of eGFR decline among the *APOL1* high-risk (2 risk alleles) and low-risk (0-1 risk alleles) groups.[5] In both of these studies, the mean age of ARIC participants at baseline (Visit 1) was 54 years.

To date, few studies have examined the natural history of *APOL1* risk variants in older age. In the Cardiovascular Health Study (mean age ~74 years), there was no difference in mean eGFR or rate of eGFR decline between the two *APOL1* risk groups; however, the *APOL1* high-risk genotypes were associated with 2-fold higher levels of albuminuria and a trend towards increased mortality risk.[7] Thus, whether the *APOL1* risk variants are associated with worse renal and clinical outcomes among individuals who survive to older age requires further clarification.

Utilizing data from ARIC Visit 5 (age of 67-90 years) onwards, we propose to study the clinical implications of having *APOL1* risk variants in older age.

5. Main Hypothesis/Study Questions:

Our main hypothesis is that among individuals who survive to older age, the *APOL1* risk variants will no longer be associated with adverse clinical outcomes.

Aim 1: To describe clinical and laboratory characteristics of ARIC participants at Visit 5 by *APOL1* genotype status.

Aim 2: To determine whether the *APOL1* high-risk genotypes are associated with worse clinical outcomes (e.g. faster estimated glomerular filtration rate [eGFR] decline, incident CKD, incident ESRD, incident and rate of hospitalization, and mortality) compared to the low-risk genotypes from Visit 5 through Visit 6.

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: We will conduct analyses of the ARIC cohort, treating Visit 5 (2011-2013) as the baseline with follow-up through Visit 6 (2016-2017).

Study Population: The study population will consist of black ARIC participants with *APOL1* genotyping and data from Visit 5. In sensitivity analyses, we will also consider white ARIC participants as a comparison group.

Exposure: The primary exposure will be *APOL1* genotype status. We will utilize a recessive genetic model in which the *APOL1* high-risk genotypes will be defined as having 2 risk alleles whereas the *APOL1* low-risk genotypes will be defined as having 0 or 1 risk allele. In sensitivity analysis, we will also consider other genetic models.

Outcomes: For Aim 1, we will examine age, sex, blood pressure, body mass index, waist circumference, diabetes, hypertension, history of heart disease, eGFR, albuminuria, LDL, HDL, triglycerides, blood sugar, hemoglobin A1c, high-sensitivity C-reactive protein (hs-CRP), general health scores, and quality of life measures (including SF12 if appropriate permissions are obtained). For Aim 2, the outcomes will be: 1) rate of eGFR decline; 2) incident CKD; 3) incident ESRD; 4) incident and rate of hospitalization; and 5) mortality. We will use both creatinine-based and cystatin-based Chronic Kidney Disease Epidemiology (CKD-EPI) Collaboration equations to estimate GFR.[8,9] In sensitivity analysis, creatinine values from heart failure or cardiovascular hospitalizations will also be included. Consistent with prior ARIC publications, incident CKD will be defined as having any one of the following: 1) an eGFR <60 ml/min/1.73 m^2 at follow-up (after Visit 5) accompanied by a ≥25% eGFR decline relative to baseline (Visit 5); 2) CKD-related hospitalization or death based on the International Classification of Diseases (ICD) 9 or 10 codes; or 3) ESRD as identified by the US Renal Data System (USRDS) registry.[10-12] At the time of ESRD onset, eGFR will be imputed as 15 ml/min/1.73 m^2. Data regarding hospitalizations and mortality will be determined from active surveillance techniques, including linkage to the National Death Index for the latter outcome.[5]

Statistical Analysis: We will use descriptive statistics, including means, medians, and proportions to compare baseline characteristics by *APOL1* genotype status. Formal testing will be performed using student's t-test or Wilcoxon rank-sum test for continuous variables and chi-squared for categorical variables. To assess the associations (e.g. hazard risk) of *APOL1* genotype status with 1) incident CKD, 2) incident ESRD, 3) incident hospitalization, and 4) mortality, a series of Cox proportional hazards models will be constructed: Model 1 will be unadjusted; Model 2 will adjust for age, sex, and European ancestry; and Model 3 will further adjust for baseline eGFR and albuminuria. Rates of hospitalization will be calculated using Poisson regression. To examine the association of *APOL1* genotype status with eGFR decline, we will fit linear mixed-effects models with random intercepts and random slopes. In these analyses, Model 1 will be unadjusted and Model 2 will adjust for age, sex, and European ancestry. We will assess for effect modification by sex, hypertension, diabetes, and inflammation (hs-CRP ≤3 mg/L vs. >3 mg/L) through the use of stratified analyses and inclusion of an interaction term with *APOL1* genotype status. In sensitivity analyses, we will also consider white ARIC participants as a comparison group. Given the low prevalence of *APOL1* alleles among individuals of European ancestry, all white participants will be imputed as having the *APOL1* low-risk genotypes.[5,13]

Proposed amendment to initial proposal:
In additional analyses, we propose to study the interactions of *APOL1* risk status with age utilizing data from Visits 1 through 7. We plan to compare incident rates of hospitalizations, ESRD, and mortality for *APOL1* high- vs. low-risk individuals within age strata (e.g., 45-54, 55-64, etc.) to better understand the significance of *APOL1* risk variants across the age spectrum. Given that Visit 7 data is now available, we plan to extend our initial analyses to also include Visit 7 data.

Limitations: We acknowledge that our proposed study has a few limitations. First, we are only using data from Visit 5 onwards. Grams *et al.* previously published on the association of *APOL1* high-risk genotypes with various clinical outcomes (including those proposed in the current

study) from Visits 1 to 5.[5] That said, duration of follow-up for the current proposed study is still long (up to 7 years). Our results will also add to our understanding of the role of *APOL1* risk variants in older age. Second, the availability of biochemical measures of kidney function will be limited to Visits 5 and 6. To address this, we will perform additional analyses incorporating eGFR that was measured during heart failure or cardiovascular hospitalizations. Third, our ability to detect associations of *APOL1* high-risk genotypes with incident and progressive CKD as well as incident hospitalization may be hindered by the competing risk of death. As such, we will consider competing risks analyses accounting for the alternative outcome of death. Last but not least, results from statistical testing that are found to be underpowered will not be reported in the manuscript, as they likely would not be meaningful.

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 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)?

Manuscript #: 2370
Race, APOL1 Risk, and eGFR Decline in the General Population. Grams ME, Rebholz CM, Chen Y, Rawlings AM, Estrella MM, Selvin E, Appel LJ, Tin A, Coresh J. *J Am Soc Nephrol*. 2016 Sep; 27(9): 2842-50.
Description: This manuscript studied the association of race and *APOL1* risk status with various clinical outcomes including incident hypertension, incident diabetes, incident cardiovascular disease, incident ESRD, hospitalizations during follow-up, all-cause mortality, and eGFR decline from Visits 1 to 5. Mean age of black participants with *APOL1* genotyping was ~54 years.

Manuscript #: 1414 (Association between MYH9 SNPs and chronic kidney disease)
APOL1 variants associate with increased risk of CKD among African Americans. Foster MC, Coresh J, Fornage M, Astor BC, Grams M, Franceschini N, Boerwinkle E, Parekh RS, Kao LWH. *J Am Soc Nephrol*. 2013 Sep; 24(9): 1481-91.
Description: This manuscript reported that the *APOL1* high-risk genotypes were associated with increased risks for incident CKD (utilizing data from Visits 1, 2, and 4) and incident ESRD (with follow-up up until 2008).

Hemostatic Factors, *APOL1* Risk Variants, and the Risk of ESRD in the Atherosclerosis Risk in Communities Study. Tin A, Grams ME, Maruthur NM, Astor BC, Couper D, Mosley TH, Fornage M, Parekh RS, Coresh J, Kao WHL. *Clin J Am Soc Nephrol.* 2015 May; 10: 784-790.
Description: This manuscript reported that associations of hemostatic factors (e.g. factor VIIIc and protein C) with ESRD risk were greater in ARIC participants who had two *APOL1* risk alleles compared to those who had one or no risk alleles.

Manuscript #: 2981
APOL1 renal-risk variants, cardiovascular disease, and all-cause mortality in African Americans. Grams M, Coresh J, Freedman B, Pajewski N, Ballew S, Sang Y.
Description: This manuscript proposes a meta-analysis of APOL1 risk and cardiovascular disease and mortality. ARIC will be one of up to 12 cohorts included. All ARIC investigators on this topic will be included in the current manuscript.

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

___ A. primarily the result of an ancillary study (list number*_____)
_X_ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* __2011.03_(Selvin for funding on visit 6 labs))

*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, approved manuscripts using 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. Genovese G, Friedman DJ, Ross MD, et al. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. *Science.* 2010;329(5993):841-845.
2. Tzur S, Rosset S, Shemer R, et al. Missense mutations in the APOL1 gene are highly associated with end stage kidney disease risk previously attributed to the MYH9 gene. *Hum Genet.* 2010;128(3):345-350.
3. Kopp JB, Nelson GW, Sampath K, et al. APOL1 genetic variants in focal segmental glomerulosclerosis and HIV-associated nephropathy. *J Am Soc Nephrol.* 2011;22(11):2129-2137.
4. Lipkowitz MS, Freedman BI, Langefeld CD, et al. Apolipoprotein L1 gene variants associate with hypertension-attributed nephropathy and the rate of kidney function decline in African Americans. *Kidney international.* 2013;83(1):114-120.
5. Grams ME, Rebholz CM, Chen Y, et al. Race, APOL1 Risk, and eGFR Decline in the General Population. *J Am Soc Nephrol.* 2016;27(9):2842-2850.
6. Foster MC, Coresh J, Fornage M, et al. APOL1 variants associate with increased risk of CKD among African Americans. *J Am Soc Nephrol.* 2013;24(9):1484-1491.
7. Mukamal KJ, Tremaglio J, Friedman DJ, et al. APOL1 Genotype, Kidney and Cardiovascular Disease, and Death in Older Adults. *Arterioscler Thromb Vasc Biol.* 2016;36(2):398-403.
8. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med.* 2009;150(9):604-612.
9. Inker LA, Schmid CH, Tighiouart H, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *The New England journal of medicine.* 2012;367(1):20-29.
10. Rebholz CM, Selvin E, Liang M, et al. Plasma galectin-3 levels are associated with the risk of incident chronic kidney disease. *Kidney international.* 2018;93(1):252-259.
11. Sumida K, Kwak L, Grams ME, et al. Lung Function and Incident Kidney Disease: The Atherosclerosis Risk in Communities (ARIC) Study. *American journal of kidney diseases : the official journal of the National Kidney Foundation.* 2017;70(5):675-685.
12. Tin A, Scharpf R, Estrella MM, et al. The Loss of GSTM1 Associates with Kidney Failure and Heart Failure. *J Am Soc Nephrol.* 2017;28(11):3345-3352.
13. O'Seaghdha CM, Parekh RS, Hwang SJ, et al. The MYH9/APOL1 region and chronic kidney disease in European-Americans. *Hum Mol Genet.* 2011;20(12):2450-2456.