ARIC Manuscript Proposal Form

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Publication Committee Review Date: **10/14/25**
ARIC Manuscript Proposal Number: **# 4598**

1.a. Full Title: Multi-omics Analysis of APOL1 Genetic Risk in Chronic Kidney Disease Development in the Atherosclerosis Risk in Communities Study

b. Abbreviated Title (Length 26 characters): Multi-omics Analysis of APOL1 Genetic Risk

2. Writing Group [please provide a middle initial if available; EX: Adam L Williams]:
Writing group members: Yang Li, Aditya Surapaneni, Pascal Schlosser, Teresa Chen, Josef Coresh, Morgan Grams

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. YL **[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 (The ARIC author should be involved enough in ARIC to be able to point the lead author to appropriate ancillary study PIs and to be able to search ARIC manuscript proposals if the lead author doesn't have the access needed to do such a search).

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3. Timeline:two years

4. Rationale:

The *APOL1* gene has two variants common in individuals of recent African ancestry: G1 (S342G and I384M) and G2 (N388-Y389 deletion), which are strongly associated with an increased risk of chronic kidney diseases (CKD). Individuals with the high-risk genotype (i.e., G1/G1, G1/G2 or G2/G2), face a significantly elevated risk of progressing from CKD to End-stage kidney disease (ESKD). However, not all individuals with these risk variants develop kidney disease or progress to ESKD, suggesting additional modifying factors. These *APOL1* risk variants have been shown to primarily disrupt podocyte function and integrity, resulting in podocyte injury, impaired endosomal trafficking, and defective autophagy, which collectively contribute to kidney disease susceptibility. In this manuscript, we aim to further our understanding of the molecular mechanisms linking *APOL1* genetic risk to kidney function.

DNA methylation plays a pivotal role in disease development by regulating gene expression and maintaining genome stability. While promoter DNA methylation of *APOL1* may influence its expression and contribute to kidney disease pathogenesis, our focus will be to utilize DNA methylation profiling technique to study the genome-wide impact of *APOL1* genetic risk on downstream molecular events, such as proteomic changes, and eventually kidney function. This DNA methylation-centered approach is based on the premise that DNA methylation changes driven by *APOL1* genetic risk could either cause or result from a cascade of molecular events involved in *APOL1*-associated kidney disease. Centering on DNA methylation can help identify genome-wide molecular events associated with *APOL1* genetic risk, as DNA methylation can modify genetic risk and regulate dynamic transcriptional changes and downstream proteomic alterations.

As higher-level molecular events in the flow of genetic information, proteome represent critical links between genotype and phenotype, serving as intermediate phenotypes that reveal the underlying disease-causing pathways in *APOL1*-related kidney disease. By integrating DNA methylome and proteome in the study of kidney function, our multi-omics study design provides a comprehensive framework to uncover the mechanistic effects of *APOL1* genetic risk on kidney disease development, to elucidate how the genetic risk is modulated in *APOL1* variant carriers, and to identify new therapeutic and interventional strategies.

5. Main Hypothesis/Study Aims:

We hypothesize that DNA methylation at specific CpG sites in peripheral blood cell DNA is associated with *APOL1* genetic risk in African American adults with CKD and that these *APOL1* genetic risk-associated methylation patterns are linked to circulating proteins. These molecular changes, in turn, influence kidney function indices, including estimated glomerular filtration rate (eGFR) and urine albumin-to-creatinine ratio (ACR), ultimately contributing to the progression from CKD to ESKD. Furthermore, we hypothesize that integrating multi-omics data will reveal distinct DNA methylation patterns, as well as proteomic signatures, that mediate the effects of *APOL1* genetic risk on kidney function.

Specific aims:
1. To investigate genome-wide DNA methylation patterns associated with *APOL1* genetic risk and their relationship with kidney function indices and ESKD in African Americans with CKD.

2. To characterize circulating proteomic changes linked to *APOL1* genetic risk-associated CpG sites and their association with kidney function indices in all participants.

3. To examine the mediating/modifying effects of *APOL1* genetic risk-associated CpGs and proteins on the relationship between *APOL1* genetic risk and kidney function indices and ESKD.

6. Design and analysis - please address the following aspects:

a) inclusion/exclusion

Inclusion:

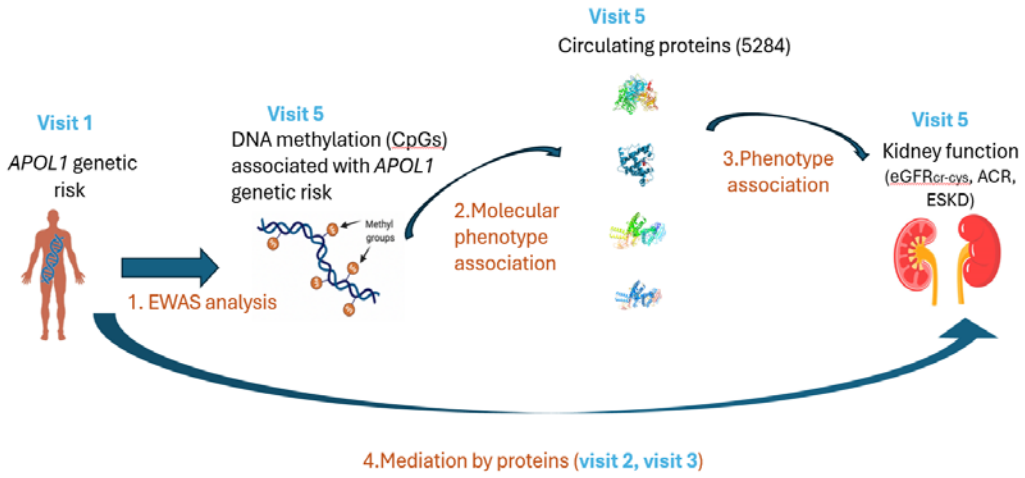
For EWAS analysis: Participants with *APOL1* genetic data (Visit 1) and DNA methylation data (Visit 5) or DNA methylation data (Visit 2) or DNA methylation data (Visit 3)

For molecular phenotype association: DNA methylation data (Visit 2, 3, 5) and blood proteomic data (Visit 2, 3, 5)

For phenotype association: blood proteomic data (Visit 2, 3, 5) and kidney function related outcomes (Visit 2, 3, 5)

For mediation analysis: *APOL1* data (Visit 1), blood proteomic data (Visit 2 and visit 3, 5), and kidney function related outcomes (Visit 2, 3 5)

b) study design



1. Cross-sectional study to perform epigenome-wide association study (Visit 5) of *APOL1* genetic risk (add on visit 2 and 3)
2. Cross-sectional study to conduct proteome-wide association study (Visit 5) of *APOL1* genetic risk-associated CpGs (Visit 5) (add on visit 2 and 3)
3. Cross-sectional study to examine association between identified CpG-associated circulating proteins (Visit 2, Visit 3, visit 5) and kidney-related outcomes

4. Longitudinal study to conduct mediation analysis of CpG-associated circulating proteins (Visit 2, Visit 3) in relation between *APOL1* genetic risk and kidney-related outcomes

c) outcome and other variables of interest with specific reference to the time of their collection

1. Primary outcomes are DNA methylation at ~850k CpG sites and circulating proteins.
2. Secondary outcomes are eGFR, ACR, and ESKD.

d) summary of data analysis

1. Descriptive statistics of demographic and clinical characteristics by *APOL1* status at visit among African American with CKD using t-test and ANOVA
2. Associations between *APOL1* genetic risk and DNA methylation among African American with CKD using multiple linear regression with adjustment for age, gender, proportions of white blood cell types estimated by DNA methylation (including CD8+T cell, CD4+T cell, natural killer cell, B cell, monocytes, and neutrophils), DNA methylation principal components (top 10)
3. Associations between *APOL1* genetic risk-associated CpGs and kidney function outcomes among all participants with CKD using multiple linear regression with adjustment for age, gender, race, center, proportions of five white blood cell types, and top 10 DNA methylation principal components
3. Associations between *APOL1* genetic risk-associated CpGs and circulating proteins among all participants with CKD using multiple linear regression with adjustment for age, gender, race, center, eGFR, proportions of five white blood cell types, and top 10 DNA methylation principal components
4. Associations between CpGs-associated circulating proteins and kidney function outcomes among all participants with CKD using multiple linear regression with adjustment for age, gender, race, and center.
5. Intra-visit correlation for CpG-associated circulating proteins (Visit 2, Visit 3, and Visit 5) among all participants with CKD using Spearman's correlation coefficient
6. Mediating effect of identified circulating proteins between *APOL1* genetic risk and kidney-related outcomesamong African Americans using R mediation package

e) Any anticipated methodologic limitations or challenges if present

1. Causal direction between DNA methylation and circulating proteins and kidney function
2. Different sample sizes for the different analyses.

f) Will the author need Limited data to complete the proposed manuscript? ☒ Yes, Limited data is needed (Provide a brief (2-3 sentences) justification for requesting PHI data) . ☐ No, De-identified data will be sufficient.

*Please note, Limited dataset access is strict and rarely provided. Limited data includes identifiable information such as dates (birthdays, visit dates, etc.). CMS, Genomic, Geocoded, Proteomics/Somalogic, and other -omic data all fall under the limited data category. De-identified data does not include dates. All dates are date adjusted to "Days since Visit 1".

7.a. Will the data be used for non-ARIC analysis or by a for-profit organization in this manuscript? (Non-ARIC analysis means that the authors are not regarded as ARIC investigators and the "ARIC author" is essentially just a facilitator rather than an integral part of the writing group.) ☐ **Yes** ☒ **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 is distributed to ARIC PIs annually, 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 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 or the "sponsoring" ARIC 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 website at: https://aric.cscc.unc.edu/aric9/proposalsearch [ARIC Website□Publications□Proposal Search]

☒ 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)?

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or does it use current [or ongoing] ancillary study data (this includes ACHIEVE)? ☐ **Yes** ☐ **No → Skip to question 12**

11.b. If yes to 11.a., is the proposal

☐ **A. primarily the result of an ancillary study**

☐ **B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables)**

11.c. If yes to 11.a., list number* __

*ancillary studies are listed by number
https://aric.cscc.unc.edu/aric9/researchers/ancillary_studies/approved_ancillary_studies [ARIC Website Ancillary Studies 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 https://aric.cscc.unc.edu/aric9/publications/policies_forms_and_guidelines [ARIC Website Publications Publication Policies, Forms, and Guidelines]. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to PubMed central.

References: ____

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