Published in final edited form as:

Kidney Int. 2018 March ; 93(3): 727–732. doi:10.1016/j.kint.2017.08.019.

APOL1 nephropathy risk variants do not associate with subclinical atherosclerosis or left ventricular mass in middle-aged black adults

Orlando M. Gutiérrez, MD, MMSc¹, Sophie Limou, PhD², Feng Lin, MS³, Carmen A. Peralta, MD, MAS³, Holly J. Kramer, MD, MPH⁴, J. Jeffrey Carr, MD, MSc⁵, Kirsten Bibbins-Domingo, MD, PhD³, Cheryl A. Winkler, PhD⁶, Cora E. Lewis, MD, MSPH¹, and Jeffrey B. Kopp, MD⁷

¹University of Alabama at Birmingham, USA

²Institute for Transplantation in Urology and Nephrology and Ecole Centrale de Nantes, France

³University of California, San Francisco, USA

⁴Loyola University, Chicago, IL, USA

⁵Vanderbilt University, Nashville, TN, USA

⁶Basic Research Laboratory, Center for Cancer Research, National Cancer Institute, Leidos Biomedical Inc., Frederick National Laboratory, Frederick, Maryland, USA

⁷Kidney Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland, USA

Abstract

Prior studies reported associations of *APOL1* nephropathy risk variants with subclinical atherosclerosis. However, these findings were limited to older individuals with high co-morbidities. To evaluate this in younger individuals, we calculated associations of *APOL1* risk variants (high risk [2 risk variants] vs. low risk [0–1 risk variant]) with prevalent, incident, or

Corresponding author: Orlando M. Gutiérrez, MD, MMSc, University of Alabama at Birmingham, ZRB 638, 1720 2nd AVE S, Birmingham, AL 35294-0006, phone: 205-996-2736, fax: 205-996-6465, ogutierrez@uabmc.edu.

DISCLOSURE

No disclosures.

The Coronary Artery Risk Development in Young Adults Study (CARDIA) is conducted and supported by the National Heart, Lung, and Blood Institute (NHLBI) in collaboration with the University of Alabama at Birmingham (HHSN268201300025C & HHSN268201300026C), Northwestern University (HHSN268201300027C), University of Minnesota (HHSN268201300028C), Kaiser Foundation Research Institute (HHSN268201300029C), and Johns Hopkins University School of Medicine (HHSN268200900041C). CARDIA is also partially supported by the Intramural Research Program of the National Institute on Aging (NIA) and an intra-agency agreement between NIA and NHLBI (AG0005). This manuscript has been reviewed by CARDIA for scientific content. OMG was supported by R01NS080850 and U01DK102730 from the NIH and by an American Heart Association Strategically Focused Network in Disparities in Cardiovascular Diseases grant (15SFDRN25620022). Dr. Bibbins-Domingo was supported by K24 DK103992. This work was supported by the Intramural Research Program, NIDDK, NIH, Bethesda, MD. This project has been funded in part with federal funds from the National Cancer Institute, National Institutes of Health, under contract HHSN26120080001E and by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

progressive coronary artery calcification, a carotid intima media thickness over the 90th percentile, and left ventricular hypertrophy in 1315 black participants of the Coronary Artery Risk Development in Young Adults (CARDIA) study. The mean age of this cohort was 44.6 years and their mean estimated glomerular filtration rate was 102.5 ml/min/1.73m². High-risk participants were found to be younger and have a higher prevalence of albuminuria than low-risk participants. In Poisson regression models adjusted for comorbidities and kidney function, the risk of prevalent coronary artery calcification (Relative Risk[95% Confidence Interval] 1.12[0.72,1.71]), the incident coronary artery calcification (1.50[0.87,2.59]), and the progression of coronary artery calcification (1.40[0.88,2.23]) did not significantly differ in high vs. low-risk participants. Furthermore, the risk of carotid intima media thickness over the 90th percentile (1.28[0.78,2.10]) and left ventricular hypertrophy (1.02[0.73,1.43]) did not significantly differ in high vs. low-risk participants in fully-adjusted models. Thus, *APOL1* risk variants did not associate with subclinical markers of atherosclerosis or left ventricular hypertrophy in middle-aged black adults with preserved kidney function.

Keywords

APOL1; cardiovascular disease; coronary artery calcification

INTRODUCTION

Two independent coding variants (G1 and G2) in the gene encoding apolipoprotein L1 (*APOL1*) are independently associated with higher prevalence and progression of chronic kidney disease (CKD) in black individuals.^{1, 2} The relationships between *APOL1* nephropathy risk variants and non-CKD outcomes have been less consistent. Several studies reported that black individuals with two *APOL1* risk variants had higher risk of incident cardiovascular disease events and death than those with zero or one risk variants,^{3, 4} whereas others showed no statistically significant associations⁵ or opposite findings.^{6, 7} Similarly with respect to subclinical markers of atherosclerosis, the Cardiovascular Health Study showed that carriage of two *APOL1* risk variants was associated with greater prevalence of peripheral vascular disease,⁴ whereas the African American-Diabetes Heart Study and Jackson Heart Study reported that carriage of *APOL1* risk variants was associated with lower prevalence of coronary artery calcification (CAC) and carotid artery calcified plaque.^{3, 7} Importantly, these latter studies were limited by the inclusion of populations that were older and had a high prevalence of co-morbid conditions, making it difficult to determine whether the association of *APOL1* risk variants with subclinical measures of atherosclerosis was independent of key risk factors, particularly kidney disease. Additionally, no studies examined the association of *APOL1* risk variants with the development or progression of CAC overtime, and only one examined the association of *APOL1* risk variants with closely linked cardiovascular phenotypes such as left ventricular hypertrophy (LVH).³ Accordingly, we examined the association of *APOL1* nephropathy risk variants with subclinical markers of atherosclerotic disease and left ventricular mass in black participants of the Coronary Artery Disease Risk In Young Adults (CARDIA) study.

RESULTS

A total of 1488 African Americans who participated in the year 20 examination had data on *APOL1* genotype. We excluded 173 with missing data on CAC, leaving a total of 1315 participants in the final analyzed sample. Of these, 12% (159) had the high-risk genotype.

The mean age of the study population was 44.6 ± 3.8 years, 39% were male, and the mean eGFR was 102.5 ± 15.9 ml/min/1.73m². Table 1 compares socio-demographic, clinical and laboratory characteristics of participants in the high nephropathy risk group (two *APOL1* risk variants) vs. the low nephropathy risk group (zero or one *APOL1* risk variants). Participants with high-risk genotypes were younger, had a higher prevalence of a urine albumin to creatinine ratio ≥ 30 mg/g, and had higher HDL-C concentrations than individuals with low-risk genotypes.

APOL1 genotype and subclinical markers of atherosclerosis

Prevalent CAC at the year 20 examination was observed in 17.9% of individuals with low-risk genotypes and 17.6% participants with high-risk genotypes (Table 2). In multivariable adjusted analyses, there were no statistically significant differences in the risk of prevalent CAC in high- vs. low-risk participants. Among participants with prevalent CAC at the year 20 examination, there was also no statistically significant difference in the Agatston score in high- risk (median 13.2, interquartile range [IQR] 2.8,77.5 units) vs. low-risk (15.6 [3.1,75.9] units) participants ($P=0.97$). Results were qualitatively unchanged in sensitivity analyses using a CAC score >10 to define prevalent CAC, when adjusting for African ancestry, or when examining differences in CAC prevalence by *APOL1* genotype using year 25 examination data (data not shown).

The development of incident CAC was observed in 13.1% of individuals with low-risk genotypes and 16.2% of individuals with high-risk genotypes. As compared to low-risk genotypes, there were no statistically significant differences in the incidence of CAC among individuals with high-risk genotypes in multivariable adjusted models (Table 2). Progression of CAC was observed in 16.6% of individuals with low-risk genotypes and 17.0% of individuals with high-risk genotypes. There were no statistically significant differences in the progression of CAC by *APOL1* genotype in multivariable adjusted models. Results were not materially changed in sensitivity analyses adjusted for time-dependent eGFR and urine albumin to creatinine ratio to account for change in kidney function between the year 20 and 25 examinations, when urine albumin to creatinine ratio was examined as a natural log-transformed variable, when examining CAC progression as the raw change in Agatston score from year 20 to 25, or when examining differences across G1/G1, G1/G2, and G2/G2 genotypes in the high-risk group (data not shown).

A composite carotid IMT $> 90^{\text{th}}$ percentile was observed in 9.9% of individuals with low-risk genotypes and 10.8% of individuals with high-risk genotypes (Table 3). In multivariable adjusted models, the risk of a composite carotid IMT $>90^{\text{th}}$ percentile did not statistically differ in participants with high-risk genotypes vs low-risk genotypes. Similarly, when examined on a continuous scale, there were no statistically significant differences in mean

composite carotid IMT in high- vs. low-risk participants (0.72 ± 0.15 vs. 0.72 ± 0.13 mm, respectively, $P=0.99$).

APOL1 genotype and left ventricular mass

LVH at the year 25 examination was present in 34.1% of individuals with low-risk genotypes and 32.1% of individuals with high-risk genotypes (Table 4). No significant differences in the risk of LVH were noted in those with high-risk genotypes as compared to those with low-risk genotypes in any of the multivariable adjusted models. Similarly, there were no statistically significant differences in mean left ventricular mass in individuals with high-risk (174.1 ± 50.1 gm) vs. low-risk (175.9 ± 54.7 gm) genotypes ($P=0.29$).

DISCUSSION

In this study of middle-aged black adults with preserved kidney function, we found no statistically significant associations of *APOL1* genotype with subclinical markers of atherosclerosis (CAC or increased carotid IMT) or LVH. These results are in contrast to prior studies that reported an association of the *APOL1* high nephropathy risk genotype with lower prevalence of CAC and carotid IMT.

The associations of *APOL1* nephropathy risk variants with cardiovascular disease have been inconsistent. The Jackson Heart Study reported that participants with two *APOL1* risk variants had greater risk of cardiovascular disease events than those with zero or one risk variants.³ Paradoxically, however, the same study showed that the high-risk *APOL1* genotype was associated with lower prevalence of CAC. The African American-Diabetes Heart Study reported that having one or two *APOL1* risk alleles was associated with lower carotid artery-calcified plaque and a trend towards lower coronary artery-calcified plaque as compared to having zero risk alleles.⁷ In contrast, the Cardiovascular Health Study found that black participants with high-risk genotypes had greater peripheral vascular disease burden (as evidenced by lower mean ankle-brachial index) than those with low-risk genotypes, but no differences in carotid IMT.⁴ In the current study of middle-aged black adults, we also found no significant differences in carotid IMT by *APOL1* genotype. Further, we found no evidence of an independent association of *APOL1* genotype with CAC.

The reasons for the differences in our findings as compared to prior studies are unclear, but could be related to the differences in the study populations examined. As compared to participants in the current study, participants of the Jackson Heart Study, African American-Diabetes Heart Study, and Cardiovascular Health Study were older and had a substantially higher prevalence of co-morbid conditions such as diabetes and CKD. It is possible that these latter factors may have altered the relationship between *APOL1* risk variants and atherosclerosis, leading to differing results. Statistical chance might also explain inconsistent results. Whether or not this is indeed the case, the results of the current study do not support a direct role of *APOL1* nephropathy risk variants in the development of subclinical atherosclerotic disease, at least in middle-aged black individuals with largely preserved kidney function.

We also found no evidence of an association of *APOL1* nephropathy risk variants with increased left ventricular mass. To our knowledge, the Jackson Heart Study is the only prior study that examined the association of *APOL1* risk variants with indexes of left ventricular structure and function. Similar to our data, Jackson Heart Study investigators found no significant differences in left ventricular mass or ejection fraction in participants with two risk alleles as compared to those with zero risk alleles.³ These data are broadly consistent with the findings of prior studies showing no associations of *APOL1* with heart disease burden, including prevalent coronary artery disease or incident heart failure.^{4, 7} Collectively, studies to date have not found a direct association of *APOL1* nephropathy risk variants with cardiac structure or function as assessed by echocardiogram, though it is possible that *APOL1* nephropathy risk variants may indirectly raise the risk of heart disease by promoting the development of kidney disease.

Our study had several strengths including a well-characterized cohort of black participants with repeated measures of CAC, and available measurements of carotid IMT and left ventricular mass obtained using standardized protocols. We also had several weaknesses. Consistent with the relatively low burden of vascular calcification among black individuals,^{8, 9} we had relatively few numbers of participants with prevalent CAC or development of incident CAC or progression of CAC. This potentially limited our power to detect more modest differences in the relative risk of these outcomes by *APOL1* genotype, which may be important given that there appeared to be a trend towards greater risk of incident and progressive CAC in those with two as compared to those with one or zero *APOL1* risk variants. For example, the sample size of this study provided sufficient power (80%) to detect as small as a 1.67-fold difference in the incidence of CAC, a larger magnitude of effect than we observed (Table 2). Future studies using pooled data or longer follow-up periods may be necessary to determine whether *APOL1* nephropathy risk variants are associated with excess risk of clinically-relevant CAC outcomes. We only had measures of eGFR and urine albumin to creatinine ratio at two time points in this study and so we could not specifically pin-point temporal associations between the decline in eGFR and the development of CAC by *APOL1* genotype. Nonetheless, adjusting for time-dependent eGFR and urine albumin to creatinine ratio did not have any effect on the effect estimates, suggesting that differences in the rate of decline in eGFR did not materially impact our findings.

In conclusion, we did not find independent associations of *APOL1* nephropathy risk variants with subclinical measures of atherosclerotic disease (CAC or carotid IMT) or LVH in middle-aged black adults with preserved kidney function. These results suggest that *APOL1* risk variants may not substantially impact the development of cardiovascular disease in younger black individuals. Whether *APOL1* nephropathy risk variants influence the development or progression of cardiovascular disease in older populations with a greater burden of co-morbidities remains an open question.

METHODS

The CARDIA study is a prospective, population-based cohort designed to examine the early determinants of cardiovascular disease. The design of this study has been detailed elsewhere.

¹⁰ Briefly, between 1985 and 1986, CARDIA recruited 5,115 black or white individuals between the ages of 18–30 years from four sites in the United States (Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA). A total of seven follow-up examinations have been conducted at years 2, 5, 7, 10, 15, 20 and 25, with retention rates of 72% for years 20 and 25. All participants provided informed consent, and the institutional review boards at each participating center have approved this study.

Genotyping

The *APOL1* G1 and G2 variant alleles were genotyped by TaqMan assays¹¹ (ABI, Foster City, CA) in black participants using samples collected at year 10.¹² The G1 haplotype is defined by rs73885319, which is in near-absolute linkage disequilibrium with the second G1 allele rs60910145, and the G2 is a 6 base pair deletion (rs71785313).¹ High nephropathy risk *APOL1* genotype status (hereafter referred to as high risk) was defined as having two risk variants, which includes homozygosity at G1 or G2 or compound heterozygosity (G1/G2). Low nephropathy risk status (hereafter referred to as low risk) was defined as carriage of zero or one risk variants. Global ancestry was estimated using the software Eigenstrat.¹³

Coronary artery calcification

CAC was assessed by computed tomography (CT) at the year 20 and 25 visits. Electron beam CT (Imatron C-150) or multidetector CT scanners (GE Lightspeed or Siemens VZ/Siemens Biograph 16) were used to obtain consecutive 2.5 -3mm-thick transverse images from the root of the aorta to the apex of the heart in two sequential electrocardiogram-gated scans.¹⁴ Experienced image analysts measured calcified plaques in the epicardial coronary arteries (left main, left circumflex, left anterior descending, and right) at a central reading center (Wake Forest University Health Sciences, Winston Salem, NC). A total calcium score, using a modified Agatston method to account for slice thickness, was calculated on an FDA-approved workstation (TeraRecon Aquarius Workstation, San Mateo, CA) for each of the two sequential scans and averaged.¹⁵ At year 20, two sequential scans were obtained and their scores averaged; at year 25 a single scan was used based on the reproducibility of prior studies and to reduce radiation exposure. Review and adjudication by an expert physician in cardiovascular imaging was performed for all participants with discordant scan pairs (year 20 examination), a change in calcium status from the previous CAC evaluation, evidence of possible surgical intervention, concerns identified by the reader, or calcium scores >200.

Carotid Intima-Media Thickness

Intima-media thickness (IMT) measurements of the common carotid artery (CCA), the carotid bifurcation (CB), and the internal carotid artery (ICA) were obtained at the year 20 visit. Carotid B-mode ultrasound examinations were conducted by trained sonographers at each field center employing a standard protocol using the GE LOGIQ 700 device.^{16, 17} Magnified longitudinal images in gray-scale of the far and near wall of the distal CCA, the CB, and the proximal ICA were obtained on the right and left sides. Images were read at the CARDIA ultrasound reading center (Tufts Medical Center, Boston, MA). The maximum IMT of each segment was defined as the mean of the maximal IMT of the near and far wall of both the left and right sides. The composite IMT measurement was calculated as an

average of all IMT measurements from the CCA, ICA, and the CB. A high composite carotid IMT was defined as $>90^{\text{th}}$ percentile.¹⁸

Left ventricular mass

Participants at the year 25 visit underwent 2-dimensional guided M-mode echocardiography in a parasternal window and 2-dimensional 4-chamber apical views following American Society of Echocardiography recommendations.^{19–21} All studies were recorded in digital format using an Artida cardiac ultrasound scanner (Toshiba Medical Systems) and interpreted by readers at the Johns Hopkins University Echocardiography Reading Center. Measurements were made from digitized images using a standard software offline image analysis system (Digisonics). Left ventricular mass index (LVMI) was calculated by dividing left ventricular mass by height raised to the power of 2.7. Left ventricular hypertrophy (LVH) was defined as defined as a LVMI $> 46.7 \text{ g/m}^{2.7}$ in women or $> 49.2 \text{ g/m}^{2.7}$ in men.²⁰

Covariates

Covariates were obtained from the year 20 examination, the baseline for this analysis. Age, sex, income, education level, smoking habits and medication use were ascertained through standardized questionnaires. Diabetes status was defined as a fasting glucose $\geq 126 \text{ mg/dL}$ or use of diabetic medications. Height and weight were measured with participants wearing light clothing and no shoes and body mass index (BMI) was calculated as kg/m^2 . After resting for five minutes, systolic and diastolic blood pressure were measured using a standard automated blood pressure measurement monitor calibrated to a random-zero sphygmomanometer. A total of three measurements were obtained with the average of the second and third measurement used. High-density lipoprotein cholesterol (HDL-C) was determined by precipitation with dextran sulfate-magnesium chloride²² and total cholesterol and triglycerides were determined enzymatically.²³ Low density lipoprotein cholesterol (LDL-C) was derived by the Friedewald equation.²⁴ Cystatin C was measured at the year 20 examination by nephelometry using the N Latex cystatin C kit (Dade Behring, now Siemens, Munich, Germany) at the University of Minnesota and were calibrated for drift as previously described.²⁵ We estimated the glomerular filtration rate (eGFR) using the most recent CKD-EPI cystatin C equation.²⁶ Urine albumin-creatinine ratios were measured as a single untimed (spot) urine sample collected at the year 20 examination. Urine albumin was measured by nephelometry, and creatinine was assessed using the Jaffe method. Urine albumin to creatinine ratio was expressed as mg per g of creatinine.

Statistical Analyses

Data are summarized as mean $\pm$ standard deviation. Baseline (year 20) characteristics were compared in high- vs. low-risk participants using Student's t-test and χ^2 -square tests as appropriate. CAC prevalence (Agatston score >0) at year 20 was compared in high- vs. low-risk participants using Poisson regression with robust variance estimation to quantify relative risk (RR) and 95% confidence intervals (95%CI).²⁷ The effect of *APOL1* risk variants were tested using a recessive model, defined as high risk genotypes compared to low risk genotypes. Model 1 was adjusted for demographic variables (age, sex, and study field center). Model 2 was adjusted for variables in Model 1 plus income, education, cardiovascular risk factors (current smoking, BMI, diabetes, systolic blood pressure, use of

anti-hypertensive medications or statins, total cholesterol, HDL, and triglycerides). Model 3 was additionally adjusted for measures of kidney function (eGFR and urine albumin to creatinine ratio). In sensitivity analyses, we examined differences in the prevalence of CAC in high- vs. low-risk participants using an Agatston score >10 to define prevalent CAC and after further adjusting models for percent African ancestry. The associations of *APOL1* genotype risk status with incidence and progression of CAC were examined using Poisson regression with a model building strategy identical to the one described above and the low-risk status serving as the referent group. Incident CAC was defined as an increase of >0 Agatston units at the year 25 examination among participants without CAC at the year 20 examination. Progression of CAC was defined as an increase of > 10 Agatston units from year 20 to year 25. In sensitivity analyses, we conducted additional analyses adjusting for time-updated urine albumin to creatinine ratio and eGFR. The association of *APOL1* risk status with the prevalence of LVH at the year 25 examination and the prevalence of carotid IMT >90th percentile at the year 20 examination was examined using a model building strategy as described above. All analyses were conducted using SAS software version 9.4 (SAS Institute, Cary, NC) and a two-tailed *P*-value < 0.05 was considered statistically significant.

References

1. Genovese G, Friedman DJ, Ross MD, et al. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. *Science*. 2010; 329:841–845. [PubMed: 20647424]
2. Parsa A, Kao WH, Xie D, et al. APOL1 risk variants, race, and progression of chronic kidney disease. *N Engl J Med*. 2013; 369:2183–2196. [PubMed: 24206458]
3. Ito K, Bick AG, Flannick J, et al. Increased burden of cardiovascular disease in carriers of APOL1 genetic variants. *Circ Res*. 2014; 114:845–850. [PubMed: 24379297]
4. Mukamal KJ, Tremaglio J, Friedman DJ, et al. APOL1 Genotype, Kidney and Cardiovascular Disease, and Death in Older Adults. *Arterioscler Thromb Vasc Biol*. 2016; 36:398–403. [PubMed: 26634651]
5. Langefeld CD, Divers J, Pajewski NM, et al. Apolipoprotein L1 gene variants associate with prevalent kidney but not prevalent cardiovascular disease in the Systolic Blood Pressure Intervention Trial. *Kidney Int*. 2015; 87:169–175. [PubMed: 25029429]
6. Ma L, Langefeld CD, Comeau ME, et al. APOL1 renal-risk genotypes associate with longer hemodialysis survival in prevalent nondiabetic African American patients with end-stage renal disease. *Kidney Int*. 2016; 90:389–395. [PubMed: 27157696]
7. Freedman BI, Langefeld CD, Lu L, et al. APOL1 associations with nephropathy, atherosclerosis, and all-cause mortality in African Americans with type 2 diabetes. *Kidney Int*. 2015; 87:176–181. [PubMed: 25054777]
8. Bild DE, Detrano R, Peterson D, et al. Ethnic differences in coronary calcification: the Multi-Ethnic Study of Atherosclerosis (MESA). *Circulation*. 2005; 111:1313–1320. [PubMed: 15769774]
9. Wagenknecht LE, Divers J, Bertoni AG, et al. Correlates of coronary artery calcified plaque in blacks and whites with type 2 diabetes. *Ann Epidemiol*. 2011; 21:34–41. [PubMed: 21130367]
10. Friedman GD, Cutter GR, Donahue RP, et al. CARDIA: study design, recruitment, and some characteristics of the examined subjects. *J Clin Epidemiol*. 1988; 41:1105–1116. [PubMed: 3204420]
11. Kopp JB, Nelson GW, Sampath K, et al. APOL1 genetic variants in focal segmental glomerulosclerosis and HIV-associated nephropathy. *J Am Soc Nephrol*. 2011; 22:2129–2137. [PubMed: 21997394]

12. Peralta CA, Bibbins-Domingo K, Vittinghoff E, et al. APOL1 Genotype and Race Differences in Incident Albuminuria and Renal Function Decline. *J Am Soc Nephrol.* 2016; 27:887–893. [PubMed: 26180129]
13. Patterson N, Price AL, Reich D. Population structure and eigenanalysis. *PLoS Genet.* 2006; 2:e190. [PubMed: 17194218]
14. Carr JJ, Nelson JC, Wong ND, et al. Calcified coronary artery plaque measurement with cardiac CT in population-based studies: standardized protocol of Multi-Ethnic Study of Atherosclerosis (MESA) and Coronary Artery Risk Development in Young Adults (CARDIA) study. *Radiology.* 2005; 234:35–43. [PubMed: 15618373]
15. Agatston AS, Janowitz WR, Hildner FJ, et al. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol.* 1990; 15:827–832. [PubMed: 2407762]
16. O'Leary DH, Polak JF, Kronmal RA, et al. Thickening of the carotid wall. A marker for atherosclerosis in the elderly? Cardiovascular Health Study Collaborative Research Group. *Stroke.* 1996; 27:224–231. [PubMed: 8571414]
17. Polak JF, Person SD, Wei GS, et al. Segment-specific associations of carotid intima-media thickness with cardiovascular risk factors: the Coronary Artery Risk Development in Young Adults (CARDIA) study. *Stroke.* 2010; 41:9–15. [PubMed: 19910544]
18. Wilkins JT, Gidding S, Liu K, et al. Associations between a parental history of premature cardiovascular disease and coronary artery calcium and carotid intima-media thickness: the Coronary Artery Risk Development In Young Adults (CARDIA) study. *Eur J Prev Cardiol.* 2014; 21:601–607. [PubMed: 23027592]
19. Gardin JM, Wagenknecht LE, Anton-Culver H, et al. Relationship of cardiovascular risk factors to echocardiographic left ventricular mass in healthy young black and white adult men and women. The CARDIA study. *Coronary Artery Risk Development in Young Adults. Circulation.* 1995; 92:380–387. [PubMed: 7634452]
20. Lang RM, Bierig M, Devereux RB, et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr.* 2005; 18:1440–1463. [PubMed: 16376782]
21. Armstrong AC, Ricketts EP, Cox C, et al. Quality Control and Reproducibility in M-Mode, Two-Dimensional, and Speckle Tracking Echocardiography Acquisition and Analysis: The CARDIA Study, Year 25 Examination Experience. *Echocardiography.* 2015; 32:1233–1240. [PubMed: 25382818]
22. Warnick GR, Benderson J, Albers JJ. Dextran sulfate-Mg²⁺ precipitation procedure for quantitation of high-density-lipoprotein cholesterol. *Clin Chem.* 1982; 28:1379–1388. [PubMed: 7074948]
23. Warnick GR. Enzymatic methods for quantification of lipoprotein lipids. *Methods Enzymol.* 1986; 129:101–123. [PubMed: 3724535]
24. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.* 1972; 18:499–502. [PubMed: 4337382]
25. Peralta CA, Vittinghoff E, Bansal N, et al. Trajectories of kidney function decline in young black and white adults with preserved GFR: results from the Coronary Artery Risk Development in Young Adults (CARDIA) study. *Am J Kidney Dis.* 2013; 62:261–266. [PubMed: 23473985]
26. Inker LA, Schmid CH, Tighiouart H, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med.* 2012; 367:20–29. [PubMed: 22762315]
27. McNutt LA, Wu C, Xue X, et al. Estimating the relative risk in cohort studies and clinical trials of common outcomes. *Am J Epidemiol.* 2003; 157:940–943. [PubMed: 12746247]

Table 1Characteristics of black CARDIA participants by *APOL1* Genotype at the year 20 examination

	High-risk genotype (N= 159)	Low-risk genotype (N=1156)	P-value
Age, mean $\pm$ SD	43.9 $\pm$ 3.7	44.7 $\pm$ 3.8	0.02
Male, n (%)	63 (39.6)	459 (39.7)	0.98
Recruitment site, n(%)			0.96
Birmingham, AL	48 (12.7)	330 (87.3)	
Chicago, IL	34 (11.5)	263 (88.6)	
Minneapolis, MN	30 (11.7)	226 (88.3)	
Oakland, CA	47 (12.2)	337 (87.8)	
Income, n (%)			0.41
<\$25,000	36 (22.6)	282 (24.4)	
\$25,000–49,000	48 (30.2)	291 (25.2)	
\$50,000	75 (47.2)	581 (50.3)	
Education, n (%)			0.69
Less than HS	8 (5.0)	73 (6.3)	
HS completed or some college	41 (25.8)	317 (27.4)	
College completed or more	110 (69.2)	762 (65.9)	
Smoking, n (%)	40 (25.2)	298 (25.8)	0.87
Diabetes, n (%)	21 (13.2)	184 (15.9)	0.38
Body mass index, mean $\pm$ SD	30.39 $\pm$ 6.65	30.83 $\pm$ 6.85	0.45
Systolic blood pressure, mean $\pm$ SD	119.9 $\pm$ 15.4	120.6 $\pm$ 16.5	0.59
Diastolic blood pressure, mean $\pm$ SD	75.6 $\pm$ 11.5	76.5 $\pm$ 11.5	0.32
eGFR, mean $\pm$ SD	101.4 $\pm$ 16.4	103.5 $\pm$ 15.4	0.12
eGFR < 60 ml/min/1.73m ² , n(%)	2 (1.3)	10 (0.9)	0.65
ACR mg/g, median [interquartile range]	6.1 [1.0,440.6]	4.9 [0.4,533.8]	0.07
ACR $\geq$ 30 mg/g, n (%)	35 (22.0)	169 (14.6)	0.02
Total cholesterol mg/dL, mean $\pm$ SD	183.9 $\pm$ 37.6	183.9 $\pm$ 34.6	0.99
HDL-C mg/dL, mean $\pm$ SD	57.3 $\pm$ 18.4	54.5 $\pm$ 16.1	0.04
LDL-C mg/dL, mean $\pm$ SD	109.0 $\pm$ 32.1	110.0 $\pm$ 33.1	0.71
Triglycerides mg/dL, mean $\pm$ SD	88.0 $\pm$ 47.2	99.4 $\pm$ 71.0	0.05
Statin use, n (%)	8 (5.0)	98 (8.5)	0.13
Anti-hypertensive use, n (%)	41 (25.8)	282 (24.4)	0.70

Abbreviations: HS, high school; eGFR, estimated glomerular filtration rate; ACR, urine albumin to creatinine ratio; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol

Data are summarized as mean $\pm$ standard deviation. Low-risk genotypes were defined as having zero or one *APOL1* nephropathy risk alleles; high-risk genotypes were defined as having two *APOL1* nephropathy risk alleles.

Table 2Prevalence, incidence and progression of coronary artery calcification by *APOL1* genotype

APOL1 genotype	n (%)	Model 1 RR (95%CI)	Model 2 RR (95%CI)	Model 3 RR (95%CI)
<i>Prevalence of CAC[*]</i>				
Low-risk genotype	207 (17.9)	ref	ref	ref
High-risk genotype	28 (17.6)	1.04 (0.70–1.54)	1.14 (0.75–1.72)	1.12 (0.73 – 1.71)
<i>Incidence of CAC[†]</i>				
Low-risk genotype	103 (13.1)	ref	ref	ref
High-risk genotype	18 (16.2)	1.29 (0.78–2.14)	1.50 (0.90–2.51)	1.50 (0.87–2.59)
<i>Progression of CAC[‡]</i>				
Low-risk genotype	158 (16.6)	ref	ref	ref
High-risk genotype	23 (17.0)	1.15 (0.74–1.78)	1.40 (0.89–2.20)	1.40 (0.88–2.23)

Abbreviations: RR, relative risk; CAC, coronary artery calcification

Low-risk genotypes were defined as having zero or one *APOL1* nephropathy risk alleles; high-risk genotypes were defined as having two *APOL1* nephropathy risk alleles.^{*} Defined as an Agatston score > 0 at the year 20 examination.[†] Defined as an increase of > 0 Agatston units at the year 25 visit among those without CAC at the year 20 visit.[‡] Defined as an increase of >10 Agatston units from year 20 visit to year 25 visit.

Model 1: adjusted for demographic variables (age [continuous], sex [M/F], study field center[categorical])

Model 2: model 1 adds socioeconomic and clinical variables (participant income [<\$25K, \$25K–49K, \$50K], participant education [< high school education, high school education, college or more], smoking [current yes or no], body mass index [continuous], systolic blood pressure [continuous], use of anti-hypertensive medications [yes or no], use of statins [yes or no], diabetes [yes or no], high-density lipoprotein cholesterol [continuous], total cholesterol [continuous], triglycerides [continuous])

Model 3: model 2 adds measures of kidney function (estimated glomerular filtration rate by CKD-EPI [continuous] and urine albumin to creatinine ratio [continuous])

Table 3

Relative risk (95% confidence interval) of a composite carotid intima media thickness >90th percentile by *APOL1* genotype at the year 20 examination

APOL1 genotype	n (%)	Model 1 RR (95%CI)	Model 2 RR (95%CI)	Model 3 RR (95%CI)
Low-risk genotype	104 (9.9)	ref	ref	ref
High-risk genotype	16 (10.8)	1.19 (0.74,1.92)	1.29 (0.79,2.12)	1.28 (0.78,2.10)

Abbreviations: RR, relative risk

Low-risk genotypes were defined as having zero or one *APOL1* nephropathy risk alleles; high-risk genotypes were defined as having two *APOL1* nephropathy risk alleles.

Model 1: adjusted for demographic variables (age [continuous], sex [M/F], study field center[categorical])

Model 2: model 1 plus socioeconomic and clinical variables (participant income [<\$25K, \$25K–49K, \$50K], participant education [< high school education, high school education, college or more], smoking [current yes or no], body mass index [continuous], systolic blood pressure [continuous], use of anti-hypertensive medications [yes or no], use of statins [yes or no], diabetes [yes or no], high-density lipoprotein cholesterol [continuous], total cholesterol [continuous], triglycerides [continuous])

Model 3: model 2 plus measures of kidney function (estimated glomerular filtration rate [continuous] and urine albumin to creatinine ratio [continuous])

Table 4

Relative risk (95% confidence interval) of left ventricular hypertrophy by *APOL1* genotype at the year 25 examination

APOL1 genotype	N (%)	Model 1 RR (95%CI)	Model 2 RR (95%CI)	Model 3 RR (95%CI)
Low-risk genotype	310 (34.1)	ref	ref	ref
High-risk genotype	43 (32.1)	0.96 (0.70–1.33)	1.02 (0.73–1.41)	1.02 (0.73–1.43)

Abbreviations: RR, relative risk

Low-risk genotypes were defined as having zero or one *APOL1* nephropathy risk alleles; high-risk genotypes were defined as having two *APOL1* nephropathy risk alleles.

Model 1: adjusted for demographic variables (age [continuous], sex [M/F], study field center[categorical])

Model 2: model 1 plus socioeconomic and clinical variables (participant income [<\$25K, \$25K–49K, \$50K], participant education [< high school education, high school education, college or more], smoking [current yes or no], body mass index [continuous], systolic blood pressure [continuous], use of anti-hypertensive medications [yes or no], use of statins [yes or no], diabetes [yes or no], high-density lipoprotein cholesterol [continuous], total cholesterol [continuous], triglycerides [continuous])

Model 3: model 2 plus measures of kidney function (estimated glomerular filtration rate [continuous] and urine albumin to creatinine ratio [continuous])