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ORIGINAL ARTICLE

APOL1 Nephropathy Risk Variants and Incident Cardiovascular Disease Events in Community-Dwelling Black Adults

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BACKGROUND: *APOL1* renal risk variants are strongly associated with chronic kidney disease in Black adults, but reported associations with cardiovascular disease (CVD) have been conflicting.

METHODS: We examined associations of *APOL1* with incident coronary heart disease (n=323), ischemic stroke (n=331), and the composite CVD outcome (n=500) in 10 605 Black participants of the REGARDS study (Reasons for Geographic and Racial Differences in Stroke). Primary analyses compared individuals with *APOL1* high-risk genotypes to *APOL1* low-risk genotypes in Cox proportional hazards models adjusted for CVD risk factors and African ancestry.

RESULTS: *APOL1* high-risk participants were younger and more likely to have albuminuria at baseline than *APOL1* low-risk participants. The risk of incident stroke, coronary heart disease, or composite CVD end point did not significantly differ by *APOL1* genotype status in multivariable models. The association of *APOL1* genotype with incident composite CVD differed by diabetes mellitus status ($P_{interaction}$=0.004). In those without diabetes mellitus, *APOL1* high-risk genotypes associated with greater risk of incident composite CVD (hazard ratio, 1.67; 95% confidence interval, 1.12–2.47) compared with those with *APOL1* low-risk genotypes in multivariable adjusted models. This latter association was driven by ischemic strokes (hazard ratio, 2.32; 95% confidence interval, 1.33–4.07), in particular, those related to small vessel disease (hazard ratio, 5.10; 95% confidence interval, 1.55–16.56). There was no statistically significant association of *APOL1* genotypes with incident CVD in subjects with diabetes mellitus. The *APOL1* high-risk genotype was associated with higher stroke risk in individuals without but not those with chronic kidney disease in fully adjusted models.

CONCLUSIONS: *APOL1* high-risk status is associated with CVD events in community-dwelling Black adults without diabetes mellitus.

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CLINICAL PERSPECTIVE

Black Americans experience significant health disparities for kidney and cardiovascular diseases. Much of the excess risk for chronic and end-stage kidney disease in Black Americans is attributable to carriage of 2 coding variants in the apolipoprotein L1 gene (*APOL1*), which are present only in persons with African ancestry; 13% of African Americans carry *APOL1* high-risk genotypes. In patients with chronic kidney disease, cardiovascular disease is the leading cause of death; however, the relationship between APOL1 renal risk variants and cardiovascular disease is unresolved and conflicting results have been reported. In this study, the authors studied 10 605 community-dwelling black participants enrolled in the REGARDS (Reasons for Geographic and Racial Differences in Stroke) to assess the association of *APOL1* renal risk variants with incident ischemic stroke and coronary heart disease. The study found that carriage of 2 *APOL1* risk alleles was not associated with cardiovascular events in individuals with chronic kidney disease or diabetes mellitus, likely because both are themselves such strong risk factors for cardiovascular disease events. However, in participants without diabetes mellitus, carriage of 2 *APOL1* risk alleles was independently associated with ischemic stroke because of small vessel occlusion and to a lesser degree, coronary heart disease. This finding may provide clues into reasons for the higher incidence rate of ischemic stroke among blacks and may also have clinical implications for screening and counseling to reduce modifiable risk factors associated with stroke and coronary heart disease in blacks without the traditional risk factors of chronic kidney disease or diabetes mellitus.

Black Americans experience significant health disparities for kidney and cardiovascular diseases (CVDs), which are only partially explained by access to medical care or other socioeconomic factors.[1–3] In patients with chronic kidney disease (CKD), CVD is the leading cause of death.[4–7] Much of the excess risk for nondiabetic CKD in Blacks is explained by 2 common coding alleles (termed G1 and G2) in *APOL1*, encoding APOL1 (apolipoprotein L1). These variants only occur on African ancestry chromosomes and extend protection against *Trypanosoma brucei*.[8–11] *APOL1* high-risk genotypes (carriage of 2 *APOL1* risk alleles) are associated with higher prevalence and progression of a spectrum of CKDs, characterized by albuminuria and reduced estimated glomerular filtration rate, each of which is risk factors for CVD.[2,5,7,12–14] The APOL1 protein is expressed in the systemic and kidney vasculature, and *APOL1* variants are associated with more severe kidney arteriosclerosis with aging and hypertension.[15,16]

The relationship between *APOL1* kidney risk variants and clinical CVD remains unresolved, with some studies reporting positive associations and others no association for prevalent or incident heart disease or stroke.[12–14,17–19] Among 1959 Black Americans in the Jackson Heart Study and 749 Black Americans in the Women's Health Initiative, *APOL1* high-risk status was associated with having higher risk for incident CVD (both cohorts), whereas individuals with *APOL1* high-risk genotypes had lower prevalence of coronary artery calcification in Jackson Heart Study participants.[14] Among 707 Black individuals, 65 years of age or older enrolled in the Cardiovascular Heart Study, *APOL1* high-risk genotypes were positively associated with subclinical peripheral atherosclerosis and incident myocardial infarction (MI).[19] However, a study of 1315 middle-aged Black adults with mostly preserved kidney function enrolled in the CARDIA (Coronary Artery Risk Development in Young Adults) failed to find an association between *APOL1* renal risk alleles and subclinical markers of atherosclerotic disease.[20]Among studies enrolling individuals with known CVD risk factors including referral for cardiac catheterization, hypertensive CKD, or hypertension, *APOL1* high-risk status did not associate with overall risk for prevalent or incident CVD.[12,13,21,22]

Given present uncertainties about the association of *APOL1* genetic risk variants with incident CVD events, we obtained *APOL1* genotypes for 10 605 Black participants enrolled in the REGARDS (Reasons for Geographic and Racial Differences in Stroke) and assessed the association of *APOL1* variants with incident ischemic stroke, coronary heart disease (CHD), and the composite of these outcomes. In addition, in prespecified analyses, we examined whether CKD, advanced age, diabetes mellitus, or sickle cell trait modified *APOL1* associations with CVD.

METHODS

The data, analytic methods, and study materials will be made available to other researchers for purposes of reproducing the results or replicating the procedure. The Institutional Review Board has reviewed and been a part for the REGARDS policies and procedures related to data access. Investigators who would like to request that data will need to first obtain information on the policies and procedures to do so. The person to contact for this information and any questions is the Publications and Presentations program manager for REGARDS, Margaret Stewart (megstewart@uab.edu).

Study Population

The REGARDS study is a population-based investigation of stroke incidence in Black and White adults ≥45 years of age. The study design has been reported elsewhere.[21]

Briefly, participants were recruited from the 48 contiguous US states and the District of Columbia. The study was designed to provide an approximately equal representation of men and women and oversampled Black individuals and persons residing in the stroke belt/buckle regions of the United States. Overall, 30 239 individuals were enrolled between January 2003 and October 2007 (42% Black, 55% women). Participants were contacted every 6 months by telephone to assess new-onset stroke, CHD, and death. Medical records were retrieved for stroke and CHD-related hospitalizations and deaths for further adjudication. The REGARDS study protocol was approved by the Institutional Review Boards governing research in human subjects at the participating centers and all participants provided informed consent.

A total of 10 872 REGARDS Black participants were genotyped for *APOL1* renal risk variants. Of those, 196 cases were missing genotype data for rs71785313, 98 cases were missing data for rs73885319, and 27 were missing data for both *APOL1* alleles, leaving 10 605 participants for the present analysis. We further excluded participants with prevalent stroke or missing data on prevalent stroke status from the analysis of incident stroke (976) and prevalent CHD or missing data on prevalent CHD status from the analysis of incident CHD (1824). Participants with prevalent stroke and prevalent CHD were also removed from the analysis of the composite CVD outcome. History of CHD at baseline was defined by a self-reported history or electrocardiographic evidence of MI or a self-reported history of a revascularization procedure. History of stroke was defined on the basis of self-report. After removing participants missing follow-up time (216) or who had a hemorrhagic stroke (38), there were 9375 participants in the stroke analysis. After removing participants missing follow-up time (191), there were 8590 participants in the CHD analysis. There were 7908 participants in the combined CVD analysis after removal of participants with a history of CHD or stroke.

Genotypic Assessment

APOL1 risk variants (G1 and G2) were genotyped using TaqMan SNP Genotyping Assays (Applied Biosystems/ThermoFisher Scientific).[9,22] *APOL1* high-risk status was defined as the presence of 2 risk alleles (G1/G1, G2/G2, or G1/G2) versus the low-risk status, defined as having 1 or 0 risk variants (G1/G0, G2/G0, G0/G0), representing a recessive model. Sickle cell trait (HbS) was genotyped as described in Naik et al.[23] In secondary analyses, an additive (0, 1, 2 risk alleles) and dominant model (2 or 1 *APOL1* risk alleles versus 0 risk alleles) were assessed. A subset of participants had available genomic array data (Illumina exome chip) to estimate population substructure (n=6714) via principal components generated using EIGENSOFT software.[24,25] In sensitivity analyses, principal components were used to adjust for African ancestry in the subset with available data.

Outcome Data

Incident ischemic stroke was investigated by retrieval of hospital records on self-report of a possible stroke/transient ischemic attack or a positive response to the Questionnaire for Verifying Stroke-Free Status during follow-up telephone contact or report by a proxy of death related to stroke. Stroke was then confirmed by a panel of neurologists according to the World Health Organization definition.[26] Events not meeting the World Health Organization definition but characterized by symptoms lasting <24 hours with neuroimaging consistent with acute infarct or hemorrhage were classified as clinical strokes. Additionally, medical records, death certificates, and autopsy reports were retrieved and reviewed to determine if a participant death was stroke-related following guidelines described. Strokes were further subclassified into etiologic subtypes of small vessel occlusion, large vessel atherosclerosis, cardioembolic, or unclassified.[27] Stroke subtype classifications were based on the stroke cause as determined during adjudication.[28–30] Data on incident stroke was available through April 1, 2016.

In addition to stroke events, CHD events including nonfatal MI or CHD death were investigated during the follow-up telephone interviews. After report of a CHD-related hospitalization or death, medical records were retrieved, and the event was adjudicated by trained clinicians following published guidelines.[31,32]CHD was confirmed by presence of signs or symptoms suggestive of ischemia; a rising or falling pattern in cardiac troponin or creatine phosphokinase-MB over 6 or more hours with a peak value greater than or equal to twice the upper limit of normal (diagnostic cardiac enzymes); and electrocardiographic changes consistent with ischemia or MI, guided by the Minnesota code. Additionally, medical records in the last year of life, death certificates and autopsy reports were collected and reviewed to determine if the death was a CHD death following published guidelines. Incident CHD events through December 31, 2013, were available for the current analyses.

A composite event was defined by the first incident CHD or ischemic stroke event during follow-up. Secondary outcomes included serum total cholesterol, HDL-C (high-density lipoprotein cholesterol), LDL-C (low-density lipoprotein cholesterol), and triglyceride levels. The serum total cholesterol, HDL-C, and triglyceride levels were measured by colorimetric reflectance spectrophotometry using the Ortho Vitros Clinical Chemistry System 950IRC instrument (Johnson & Johnson Clinical Diagnostics, New Brunswick, NJ). LDL-C was further calculated from these 3 measures using the Friedewald equation.[33,34]

Data Collection at Baseline

Data on covariates of interest were collected during a baseline in-home visit (including the collection of a blood and spot urine sample) and a separate computer-assisted telephone interview. Information on age, smoking status, lipid-lowering drugs, and antihypertensive medication use was collected by self-report. Systolic and diastolic blood pressure were defined as the average of 2 seated blood pressure measures taken after a 5-minute rest. Diabetes mellitus was defined as fasting serum glucose ≥126 mg/dL, nonfasting serum glucose ≥200 mg/dL, or use of antidiabetes mellitus medications. Serum creatinine was calibrated to an international isotope dilution mass spectroscopic-traceable standard measured by colorimetric reflectance spectrophotometry. We calculated estimated glomerular filtration rate using the CKD-Epidemiology Collaboration equation.[35] Albumin and creatinine were measured using the random spot urine specimen by nephelometry (BN ProSpec Nephelometer, Dade Behring, Marburg, Germany) and Modular-P chemistry analyzer (Roche/Hitachi, Indianapolis, IN), respectively. Spot urine albumin-to-creatinine ratio was calculated in mg/g. Abnormal albumin-to-creatinine ratio was defined as urine albumin-to-creatinine

ratio >30 mg/g. Serum hsCRP (high-sensitivity C-reactive protein) was measured using a high-sensitivity particle-enhanced immunonephelometric assay.

Statistical Analyses

We compared participant characteristics by *APOL1* risk allele count (0, 1, or 2) using the χ^2 test for categorical variables and ANOVA for continuous variables. We calculated incidence rates and 95% confidence intervals (CIs) of each outcome for each risk category (0, 1, or 2). After confirming the proportionality assumption, Cox proportional hazards models were used to assess the association of *APOL1* high-risk versus low-risk status (recessive model) with incident events. The follow-up time was measured from the baseline interview date to occurrence of the event or last follow-up. We generated hazard ratios (HRs) from 3 models including a crude model, a second model adjusted for age, sex, smoking (former, current, or never), hypertension (defined as baseline systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or self-reported use of hypertension medications), lipid-lowering medications, and diabetes mellitus, and a third model adjusted for variables in model 2 as well as principal components for ancestry in the subset of individuals with available data. Secondary analyses considered additive and dominant models for testing the association of *APOL1* risk variants with CVD outcomes. Stratified models were used to assess effect modification of the relationship between *APOL1* genotypes and CVD outcomes by diabetes mellitus status, the presence or absence of CKD, baseline age ≥65 compared with <65 years, and sickle cell trait status. Unstratified models that included an interaction term were used to evaluate statistical significance. Analyses of fasting lipids were assessed in crude and multivariable adjusted linear regression models. All analyses were performed using SAS v. 9.4.

RESULTS

Study Population

Of 10 605 participants, 4420 had 0 *APOL1* risk alleles, 4839 had 1 risk allele (2989 had the G1/0 geno-

Table 1. Baseline Characteristics by Category of *APOL1* Nephropathy Risk Variant Status

Variables	*APOL1* Risk Variants			*P* Value
	0	1	2	
N (%)	4420 (41.68)	4839 (45.63)	1346 (12.69)	
Male sex, N (%)	1701 (38.48)	1888 (39.02)	512 (38.04)	0.76
Age, mean (SD)	64.06 (9.24)	64.14 (9.35)	63.36 (8.85)	0.02
Smoking, N (%)				0.10
Current	774 (17.58)	873 (18.12)	213 (15.96)	
Never	1961 (44.54)	2183 (45.31)	644 (48.24)	
Past	1688 (37.88)	1762 (36.57)	478 (35.81)	
Prevalent stroke	357 (8.10)	363 (7.53)	101 (7.52)	0.56
Prevalent coronary heart disease	679 (15.67)	734 (15.50)	194 (14.70)	0.69
Diabetes mellitus, N (%)	1266 (28.93)	1454 (30.40)	399 (29.84)	0.30
Dyslipidemia, N (%)	1310 (53.58)	2631 (55.30)	748 (56.16)	0.13
Systolic blood pressure, mean (SD)	130.52 (17.40)	131.19 (17.35)	131.34 (17.39)	0.11
Diastolic blood pressure, mean(SD)	78.34 (10.07)	78.59 (10.23)	78.96 (9.93)	0.12
Hypertension medications N (%)	2905 (66.51)	3264 (68.16)	914 (68.93)	0.13
Lipid-lowering medications, N (%)	1310 (29.94)	1500 (31.34)	437 (32.71)	0.11
Triglycerides, mean (SD)	115.59 (84.46)	112.94 (65.01)	114.27 (65.20)	0.24
Total cholesterol, mean (SD)	192.98 (40.92)	193.47 (41.89)	195.65 (39.50)	0.01
LDL-cholesterol, mean (SD)	116.01 (36.24)	117.29 (37.15)	118.59 (35.00)	0.051
HDL-cholesterol, mean (SD)	53.00 (15.48)	53.43 (16.20)	54.20 (16.77)	0.049
eGFR <60 mL/min per 1.73 m^2, N (%)*	563 (12.74)	604 (12.48)	190 (14.12)	0.28
ACR ≥30 g/mg, N (%)†	784 (17.74)	882 (18.32)	296 (21.99)	0.002
Chronic kidney disease, N (%)‡	1135 (25.7)	1242 (25.7)	396 (29.4)	0.01
hsCRP, mean (SD)§	5.62 (9.15)	5.91 (11.53)	5.62 (8.02)	0.33

Results are presented as means (SE) or frequencies. *P* values denote differences between *APOL1* categories (0 vs 1 vs 2 risk alleles). APOL1 indicates apolipoprotein L1; HDL, high-density lipoprotein; and LDL, low-density lipoprotein.

*eGFR indicates estimated glomerular filtration rate.

†ACR indicates urine albumin-to-creatinine ratio.

‡Chronic kidney disease defined as an eGFR <60 mL/min per 1.73 m2 or an ACR ≥30 mg/g.

§hsCRP indicates high-sensitivity C-reactive protein.

Table 2. Incidence Rates (95% Confidence Intervals) for Ischemic Stroke, Coronary Heart Disease, and the Composite Outcome Per 1000 Person-Years of Follow-Up According to *APOL1* Risk Variant Status

	APOL1 Risk Alleles		
	0	1	2
Ischemic stroke*	3.38 (2.83–4.03)	4.07 (3.49–4.74)	4.09 (3.06–5.46)
Coronary heart disease†	4.39 (3.91–5.23)	4.84 (4.13–5.68)	5.19 (3.89–6.93)
Composite events‡	7.14 (6.20–8.24)	8.39 (7.39–9.53)	9.01 (7.16–11.33)

*Excludes prevalent stroke, hemorrhagic, and nonadjudicated strokes.
†Excludes prevalent coronary heart disease.
‡Excludes prevalent stroke, hemorrhagic strokes, nonadjudicated strokes, and prevalent coronary heart disease.

type and 1850 the G2/0 genotype), and 1346 had 2 risk alleles (530 were G1 homozygotes, 205 were G2 homozygotes, and 611 were compound G1/G2 heterozygotes).

Table 1 compares demographic, clinical, and laboratory characteristics of participants by a number of *APOL1* G1/G2 risk variants. The participants with higher numbers of risk variants were younger, had higher total cholesterol and HDL-C concentrations, and were more likely to have albuminuria at baseline.

Association of *APOL1* Risk Status With Incident CVD

There were 331 ischemic stroke events, 323 CHD events, and 500 composite events. Crude incidence rates of the combined CVD end point and individual components of the end point by *APOL1* genotype are depicted in Table 2. In general, incident CVD rates numerically increased with carriage of 1 or 2 *APOL1* risk variants.

HRs of CVD events by *APOL1* genotype (comparing 2 versus 0 or 1 risk variants) are shown in Table 3. In crude and multivariable adjusted models, there were no statistically significant differences in the risk of incident stroke, CHD, or the composite CVD end point in individuals with 2 as compared with 0 or 1 *APOL1* risk variants. The results did not differ when analyses were repeated using additive or dominant models (Tables I and II in the Data Supplement) or when comparing individuals homozygous for G1 or G2 to those with 0 risk variants (Tables III and IV in the Data Supplement).

The association of *APOL1* genotype with incident composite CVD differed by diabetes mellitus status with a *P* value for interaction of 0.004 (Table 4). Among those without diabetes mellitus, individuals with 2 *APOL1* risk variants had a higher risk of incident combined CVD than individuals with 0 or 1 *APOL1* risk variants in the crude model (HR, 1.42; 95% CI, 1.05–1.92) and the multivariable adjusted model (HR, 1.48; 95% CI, 1.09–2.01). The results strengthened when adjusted for ancestry (HR, 1.67; 95% CI, 1.12–2.47), indicating that *APOL1* variants and not African ancestry per se is driving the association. When examining individual components of the composite outcome, the magnitude and strength of the association were greatest for incident stroke events (HR, 2.32; 95% CI, 1.33–4.07). In contrast, among individuals with diabetes mellitus at the baseline visit, there was no significant association of *APOL1* genotype with risk of incident CVD in any of the models, and the direction of the association was opposite to what was observed for individuals without diabetes mellitus. The results were similar, but the associations were weaker when analyses were repeated using additive and dominant models (Tables V and VI in the Data Supplement, respectively).

Among those with normal kidney function at baseline, individuals with 2 *APOL1* risk variants had a higher hazard of incident stroke (HR, 1.79; 95% CI, 1.04–3.07) than individuals with 0 or 1 *APOL1* risk variants in the fully adjusted model including ancestry (Model 2; Table 5). In contrast, risks for incident CHD or composite events did not significantly differ by *APOL1* genotype in this group. The associations of *APOL1* high-risk genotype with stroke in those with normal kidney function were qualitatively similar in additive and dominant models (Tables VII and VIII in the Data Supplement). Among those with CKD at baseline, there were no statistically significant associations of *APOL1* variants with

Table 3. HRs of Incident Ischemic Stroke, CHD, and the Composite Outcome Comparing 2 vs 0 or 1 *APOL1* Risk Variants (Recessive Model)

	Events/Total N (%)	Crude, HR (95% CI)	Model 1, HR (95% CI)	Model 2, HR (95% CI)
Ischemic stroke*	331/9375 (3.5)	1.09 (0.80–1.49)	1.14 (0.83–1.57)	1.34 (0.85–2.14)
CHD†	323/8590 (3.8)	1.14 (0.82–1.60)	1.15 (0.82–1.62)	0.97 (0.63–1.50)
Composite events‡	500/7908 (6.4)	1.14 (0.89–1.46)	1.16 (0.90–1.49)	1.15 (0.82–1.61)

Model 1 adjusted for age, sex, smoking, hypertension, lipid-lowering medications and diabetes mellitus (events/N: stroke=322/9140; CHD=317/8381; composite=490/7719). Model 2 adjusted for variables in model 1 plus ancestry principal components (events/N: stroke=127/6324; CHD=221/5894; composite=273/5435). CHD indicates coronary heart disease; CI, confidence interval; and HR, hazard ratio.
*Excludes prevalent stroke, hemorrhagic, and nonadjudicated strokes.
†Excludes prevalent coronary heart disease.
‡Excludes prevalent stroke, hemorrhagic strokes, nonadjudicated strokes, and prevalent coronary heart disease.

Table 4. HRs (95% CIs) of Incident Ischemic Stroke, CHD, and the Composite Outcome Comparing 2 vs 0 or 1 *APOL1* Risk Variants (Recessive Model), Stratified by Diabetes Status

	Events/Total N (%)	Crude, HR (95% CI)	Model 1, HR (95% CI)	Model 2, HR (95% CI)
No diabetes mellitus				
Ischemic stroke*	197/6621 (3.0)	1.36 (0.93–1.98)	1.46 (0.99–2.14)	2.32 (1.33–4.07)
CHD†	174/6173 (2.8)	1.37 (0.92–2.04)	1.40 (0.94–2.09)	1.31 (0.81–2.10)
Composite events‡	297/5755 (5.1)	1.42 (1.05–1.92)	1.48 (1.09–2.01)	1.67 (1.12–2.47)
Diabetes mellitus				
Ischemic stroke*	131/2660 (4.9)	0.74 (0.42–1.28)	0.76 (0.44–1.33)	0.55 (0.22–1.37)
CHD†	148/2329 (6.4)	0.85 (0.51–1.41)	0.78 (0.46–1.34)	0.53 (0.24–1.14)
Composite events‡	200/2072 (9.7)	0.76 (0.49–1.18)	0.76 (0.48–1.19)	0.52 (0.26–1.03)

Model 1 adjusted for age, sex, smoking, hypertension, and lipid-lowering medications (diabetes mellitus, events/N: stroke=129/2620; CHD=144/2296; composite=196/2044; no diabetes mellitus, events/N: stroke=193/6520; CHD=173/6085; composite=294/5675). Model 2 adjusted for variables in model 1 plus ancestry principal components (diabetes mellitus, events/N: stroke=60/1861; CHD=96/1669; composite=113/1489; no diabetes mellitus, events/N: stroke=67/4463; CHD=125/4225; composite=160/3946). $P_{interaction}$ for diabetes mellitus on the association of *APOL1* high-risk status with incident stroke, CHD, and the composite outcome in the fully adjusted model (model 2) were 0.02, 0.07, and 0.008, respectively. CHD indicates coronary heart disease; CI, confidence interval; and HR, hazard ratio.

*Excludes prevalent stroke, hemorrhagic, and nonadjudicated strokes.

†Excludes prevalent coronary heart disease.

‡Excludes prevalent stroke, hemorrhagic strokes, nonadjudicated strokes, and prevalent coronary heart disease.

CVD outcomes in any model (Table 5; Tables VII and VIII in the Data Supplement). Results did not materially change when stratified by estimated glomerular filtration rate (< versus ≥60 mL/min per 1.73 m2) or albumin-to-creatinine ratio (≥ versus <30 mg/g), separately (Tables IX and X in the Data Supplement).

Tables XI through XIV in the Data Supplement show models stratified by age and sickle cell trait status. In general, there were no statistically significant differences in the association of *APOL1* risk variants with incident CVD events by strata of age or sickle cell trait in fully adjusted models including ancestry principal components.

APOL1 Variants and Ischemic Stroke Subtypes

Associations of *APOL1* risk variants with ischemic stroke subtypes are presented in Table XV in the Data Supplement. Carriage of the high-risk genotype was associated with greater risk of strokes because of small vessel disease in fully adjusted models (HR, 3.74; 95% CI, 1.61–8.65). When stratified by diabetes mellitus status, the magnitude and strength of this association were greater in individuals without diabetes mellitus (no diabetes mellitus: HR, 5.10; 95% CI, 1.55–16.56; diabetes mellitus: HR, 2.67; 95% CI, 0.79–9.04). No statistically significant associations of high-risk geno-

Table 5. HRs (95% CIs) of Incident Ischemic Stroke, CHD, and the Composite Outcome Comparing 2 vs 0 or 1 *APOL1* Risk Variants (Recessive Model), Stratified by CKD Status

	Events/Total N (%)	Crude, HR (95% CI)	Model 1, HR (95% CI)	Model 2, HR (95% CI)
No CKD				
Ischemic stroke*	211/7098 (3%)	1.26 (0.86–1.84)	1.35 (0.92–1.98)	1.79 (1.04–3.07)
CHD†	186/6557 (3%)	1.17 (0.77–1.77)	1.19 (0.78–1.80)	0.97 (0.56–1.70)
Composite events‡	300/6125 (5%)	1.17 (0.85–1.62)	1.22 (0.88–1.70)	1.20 (0.77–1.87)
CKD				
Ischemic stroke*	158/2315 (7%)	0.79 (0.49–1.26)	0.83 (0.51–1.35)	0.77 (0.36–1.61)
CHD†	137/2033 (7%)	0.95 (0.59–1.53)	0.99 (0.60–1.62)	0.99 (0.55–1.79)
Composite events‡	200/1783 (11%)	0.97 (0.6–1.43)	1.04 (0.70–1.56)	1.10 (0.65–1.85)

Model 1 adjusted for age, sex, smoking, hypertension, lipid-lowering medications, and diabetes mellitus (CKD, events/N: stroke=152/2228; CHD=133/1953; composite=194/1712; no CKD, events/N: stroke=205/6947; CHD=184/6428; composite=296/6007). Model 2 adjusted for variables in model 1 plus ancestry principal components (CKD, events/N: stroke=66/1561; CHD=98/1405; composite=112/1230; no CKD, events/N: stroke=86/4788; CHD=123/4489; composite=161/4205). $P_{interaction}$ for CKD on the association of *APOL1* high-risk status with incident stroke, CHD, and the composite outcome in the fully adjusted model (model 2) were 0.04, 0.90, and 0.69, respectively. CKD (defined as an estimated glomerular filtration rate <60 mL/min per 1.73 m2 or a spot urine albumin-to-creatinine ratio ≥30 mg/g). CHD indicates coronary heart disease; CI, confidence interval; CKD, chronic kidney disease; and HR, hazard ratio.

*Excludes prevalent stroke, hemorrhagic, and nonadjudicated strokes.

†Excludes prevalent coronary heart disease.

‡Excludes prevalent stroke, hemorrhagic strokes, nonadjudicated strokes, and prevalent coronary heart disease.

types with other stroke subtypes were noted in multivariable adjusted models.

Association of *APOL1* Variants With Lipid Profiles

Since APOL1 is a constituent of HDL, and HDL-C concentrations correlate with cardiovascular risk, we analyzed the associations of *APOL1* risk variants with lipid measures under a recessive model (Table XVI in the Data Supplement). HDL-C and total cholesterol concentrations were significantly higher in individuals with 2 as compared with 0 or 1 *APOL1* risk variants in models adjusted for age, sex, smoking, use of lipid-lowering medications, hypertension, and diabetes mellitus. However, these associations were attenuated and no longer statistically significant after adjustment for ancestry in the subset of individuals with available data. Similar results were obtained when examining additive or dominant models (Table XVII and XVIII in the Data Supplement).

DISCUSSION

In this study of community-dwelling Black adults, we assessed the relationship between *APOL1* risk variants and incident ischemic stroke, CHD and a composite outcome comprising these clinical events. We observed a trend for higher incidence rates for stroke, CHD, and composite events with increased with greater carriage of *APOL1* risk alleles. Further, the association of *APOL1* nephropathy risk variants with CVD risk differed by diabetes mellitus status, such that *APOL1* high-risk status was independently associated with higher risk for composite CVD events, primarily driven by ischemic stroke, in patients without diabetes mellitus; whereas in patients with diabetes mellitus and *APOL1* high-risk status, the risk of stroke, CHD, and composite events was not statistically significant and in the opposite direction. We also found a less robust, but still significant, an association of *APOL1* high-risk status with incident stroke in a subgroup analysis of subjects without CKD. Our findings suggest that conflicting results among prior studies for *APOL1* associations with CVD may be because of effect modification by diabetes mellitus and CKD, each of which is a known independent risk factor for CVD.

The association of *APOL1* with subclinical and clinical CVD has been inconsistent, showing positive association (risk), negative association (protection), or no association for subclinical atherosclerosis and clinical CVD outcomes. Differences in the direction of association have been attributed to the complexity of shared and reciprocal risk factors for CVD and CKD and the inherent difficulties in adequately adjusting for confounding by severity of kidney disease, all of which are strongly associated with *APOL1* high-risk status and are risk factors for CVD.[6,18,36,37] Positive *APOL1* high-risk genotype associations with incident CVD have been evident in some (Jackson Heart Study, Women's Health Initiative, and Cardiovascular Health Study) but not all (Atherosclerosis Risk in Communities) population-based cohort studies.[14,19,38] In contrast, in studies enrolling participants with known risk factors for CVD, including referral for cardiac catheterization, hypertension, and CKD, the association between *APOL1* high-risk status and incident CVD was weak or absent, perhaps because it was masked by the stronger risk conferred by underlying morbidities.[12,13,39] Relative proportions of European and African ancestry are also potential confounders. Europeans are at greater risk for atherosclerosis, and similarly, increasing European ancestry in Black Americans is associated with more coronary calcification and atherosclerosis, which are predictors of CVD.[40,41]

In an effort to determine whether CKD, advanced age, diabetes mellitus, or sickle cell trait might be modifiers of *APOL1* associations with CVD, we performed subgroup analyses. *APOL1* high-risk status was not associated with CVD in subgroups with CKD or diabetes mellitus at baseline. However, among patients without diabetes mellitus, *APOL1* high-risk status was associated with greater risk for the composite outcome in the crude model. Further, the magnitude and strength of the association became stronger in the fully adjusted model accounting for African ancestry, primarily driven by stroke. Additionally, *APOL1* high-risk genotypes were significantly associated with 79% greater hazard for incident stroke in patients without CKD in the fully adjusted model. These results indicate that the *APOL1* high-risk genotype is an independent risk factor for ischemic stroke and, to a lesser degree, CHD, among individuals without diabetes mellitus or CKD. It is also notable that *APOL1* high-risk genotypes were most strongly associated with ischemic strokes because of small vessel occlusion, particularly in individuals without diabetes mellitus. If replicated in future studies, these results may provide novel insights into reasons for racial differences in the incidence of strokes caused by small vessel disease.

The notable interaction between diabetes mellitus and *APOL1* for incident CVD events was unexpected, although consistent with CKD studies which report positive *APOL1* associations for nondiabetic kidney disease but not for diabetic kidney disease.[9,42,43] Additionally, *APOL1* high-risk status was associated with greater hemodialysis survival rates, mainly attributed to CVD, for dialysis patients with nondiabetic end-stage kidney disease, but not in patients with diabetic end-stage kidney disease.[44] Our results, together with others, suggest that diabetes mellitus may attenuate *APOL1*

penetrance. High concentrations of glucose result in oxidative imbalance and downregulation of autophagic pathways required for cell homeostasis.[18,45] *APOL1* variant proteins disrupt normal trafficking of endosomal vesicles in kidney cells causing reduced autophagic flux, oxidative stress, and cytoxicity.[46–48] The pathophysiological mechanisms underlying the putative diabetes mellitus interaction with *APOL1* risk variants warrants further investigation.

To determine whether the *APOL1* associations with CVD followed a recessive model, as it does for nondiabetic CKD, we tested the additive and dominant genetic models in the subgroup analyses. We found that *APOL1* high-risk status was significantly associated with stroke and composite events under the additive, but not the dominant genetic model, with each variant allele increasing risk for ischemic stroke by 54%. Ito et al[14] previously reported that the G2 deletion allele may have greater penetrance (effect size) than the missense G1 variants; however, we found no evidence of this in our study. A more recent study of older patients enrolled in the Cardiovascular Health Study also found no differences in penetrance sizes for the G1 and G2 renal risk alleles with MI.[19]

To investigate whether lipids may play a role in the association of *APOL1* genotype with CVD outcomes, we examined the association of *APOL1* with available lipid parameters in REGARDS. *APOL1* risk alleles were associated with total cholesterol, HDL-C, and LDL-C in crude models for additive, dominant, and recessive models, but adjusting for African ancestry abrogated the associations. These results argue against dyslipidemia playing a primary role in the association between *APOL1* risk variants and CVD risk. Nonetheless, a previous study of Black participants enrolled in the REGARDS reported that increasing numbers of *APOL1* were associated with higher circulating levels of small HDL particles, which may contribute to greater risks of CVD in study participants carrying *APOL1* risk variants.[49] Mechanistically, *APOL1* protein isoform association with small HDL complexes rather than overall HDL-C levels might be more relevant to CVD risk.[50]

The strengths of our study include the large, community-based sample of Black Americans with extensive data on cardiovascular risk factors, with genotyping for *APOL1* renal risk variants, sickle cell trait, and Genome Wide Association Study data for 6714 participants to correct for ancestry substructure. By limiting our study to only incident ischemic CVD events, we reduced phenotype heterogeneity. Our study does have limitations. We did not have ancestry informative markers on the entire cohort, which might have strengthened the *APOL1* associations. In addition, we did not have follow-up measures of kidney function, precluding us from determining whether associations of *APOL1* risk variants with CVD outcomes was at least partially explained by a faster decline in estimated glomerular filtration rate or a greater increase in albuminuria over time.

In conclusion, in a large, community-based study of Black Americans across the United States, *APOL1* was independently associated with CVD in nondiabetics, and to a lesser degree, in persons without CKD, and these associations were strengthened after correction for African ancestry. This study highlights the complexity of *APOL1* association with clinical CVD and the difficulties in disentangling multiple interactive factors that contribute to CVD.

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Disclosures

None.

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APOL1 Nephropathy Risk Variants and Incident Cardiovascular Disease Events in Community-Dwelling Black Adults

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Supplemental Material

Supplemental Table 1. Association of *APOL1* risk alleles with incident cardiovascular outcomes, additive model.

Outcomes	Events/Total N	Crude HR (95% CI)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
Stroke*	331/9375	1.12 (0.96-1.31)	1.13 (0.97-1.33)	1.16 (0.90-1.49)
Coronary heart disease †	323/8590	1.09 (0.93-1.28)	1.07 (0.91-1.26)	1.00 (0.82-1.22)
Composite Events ‡	500/7908	1.13 (0.95-1.28)	1.13 (0.99-1.29)	1.09 (0.91-1.30)

Shown are the hazard ratios (HR) (95% confidence interval) per *APOL1* risk variant for stroke, coronary heart disease, and composite events. * Excludes prevalent stroke, and hemorrhagic and non-adjudicated strokes; † Excludes prevalent coronary heart disease; ‡ Excludes prevalent stroke, hemorrhagic strokes, non-adjudicated strokes, and prevalent coronary heart disease.

Model 1 adjusted for age, sex, smoking, hypertension, lipid medications and diabetes. [Events/N: Stroke = 322/9140; CHD = 317/8381; Composite = 490/7719]
Model 2 adjusted for variables in Model 1 plus principal components of ancestry. [Events/N: Stroke = 127/6324; CHD =221/5894; Composite = 273/5435]

Supplemental Table 2. Association of *APOL1* risk alleles with incident cardiovascular outcomes, dominant model.

Outcomes	Events/Total N	Crude HR (95% CI)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
Stroke *	331/9375	1.20 (0.96-1.51)	1.20 (0.96-1.50)	1.13 (0.79-1.63)
Coronary heart disease †	323/8590	1.12 (0.90-1.40)	1.09(0.87-1.37)	1.02 (0.77-1.33)
Composite Events ‡	500/7908	1.19 (0.99-1.42)	1.18 (0.99-1.42)	1.10 (0.86-1.41)

Shown are the hazard ratios (HR) (95% confidence interval) for stroke, coronary heart disease, and composite events comparing individuals with 1 or 2 *APOL1* risk variants to 0 risk variants. *Excludes prevalent stroke, and hemorrhagic and non-adjudicated strokes; † Excludes prevalent coronary heart disease; ‡ Excludes prevalent stroke, hemorrhagic strokes, non-adjudicated strokes, and prevalent coronary heart disease.

Model 1 adjusted for age, sex, smoking, hypertension, lipid medications and diabetes. [Events/N: Stroke = 322/9140; CHD = 317/8381; Composite = 490/7719]
Model 2 adjusted for variables in Model 1 plus principal components of ancestry. [Events/N: Stroke = 127/6324; CHD =221/5894; Composite = 273/5435]

Supplemental Table 3. Association of *APOL1* risk alleles with incident cardiovascular outcomes, categorical model (1 G1 vs. 0, and 2 G1 vs. 0).

Outcomes	Risk Allele	Events/total N	Crude HR (95% CI)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
Stroke *	0	232/6943	Ref	Ref	Ref
	G1/0		1.13(0.86-1.48)	1.09(0.83-1.44)	0.94(0.60-1.50)
	G1/G1		1.19(0.73-1.96)	1.27(0.77-2.08)	0.93(0.40-2.20)
Coronary heart disease †	0	241/6377	Ref	Ref	Ref
	G1/0		1.11 (0.84-1.45)	1.06(0.81-1.39)	1.07(0.78-1.48)
	G1/G1		1.56(1.01-2.41)	1.55(1.00 -2.40)	1.44(0.64-2.05)
Composite Events ‡	0	362/5876	Ref	Ref	Ref
	G1/0		1.15(0.93-1.43)	1.13(0.91-1.41)	1.08(0.81-1.45)
	G1/G1		1.42(0.98-2.05)	1.44(1.00-2.09)	1.25(0.74-2.09)

Shown are the hazard ratios (HR) (95% confidence interval) for stroke, coronary heart disease, and composite events. * Excludes prevalent stroke, and hemorrhagic and non-adjudicated strokes; † Excludes prevalent coronary heart disease; ‡ Excludes prevalent stroke, hemorrhagic strokes, non-adjudicated strokes, and prevalent coronary heart disease.

Model 1 adjusted for age, sex, smoking, hypertension, lipid medications and diabetes.[Events/N: Stroke = 225/6769; CHD = 237/6219; Composite = 354/5734]
Model 2 adjusted for variables in Model 1 plus principal components of ancestry. [Events/N: Stroke = 85/4685; CHD = 170/4383; Composite = 201/4043]

Supplemental Table 4. Association of *APOL1* G2 variant with incident cardiovascular disease, categorical model (1 G2 vs. 0, and 2 G2 vs. 0)

Outcomes	Risk Allele	Events/total N	Crude	Model 1	Model 2
			HR (95% CI)	HR (95% CI)	HR (95% CI)
Stroke *	0	200/5728	Ref	Ref	Ref
	G2/0		1.32(1.01-1.80)	1.33(0.99-1.80)	1.21(0.74-1.98)
	G2/G2		1.22(0.57-2.62)	1.26(0.59-2.69)	1.46(0.52-4.08)
Coronary heart disease †	0	187/5227	Ref	Ref	Ref
	G2/0		1.09 (0.79-1.48)	1.07(0.78-1.47)	0.91(0.61-1.36)
	G2/G2		0.69(0.26-1.87)	0.74(0.27-2.00)	0.72(0.23-2.28)
Composite Events ‡	0	293/4824	Ref	Ref	Ref
	G2/0		1.21(0.94-1.55)	1.21(0.95-1.56)	1.03(0.73-1.47)
	G2/G2		1.13(0.59-2.13)	1.23(0.65-2.32)	1.07(0.46-2.46)

Shown are the hazard ratios (HR) (95% confidence interval) for stroke, coronary heart disease, and composite events. * Excludes prevalent stroke, and hemorrhagic and non-adjudicated strokes; † Excludes prevalent coronary heart disease; ‡ Excludes prevalent stroke, hemorrhagic strokes, non-adjudicated strokes, and prevalent coronary heart disease.

Model 1 adjusted for age, sex, smoking, hypertension, lipid medications and diabetes. [Events/N: Stroke = 195/5591; CHD = 184/5104; Composite =287/4714]
Model 2 adjusted for variables in Model 1 plus principal components of ancestry. [Events/N: Stroke = 76/3831; CHD = 126/3563; Composite = 156/3289]

Supplemental Table 5. Association of *APOL1* risk alleles with incident cardiovascular outcomes, stratified by diabetes status, additive model.

Outcomes	Events/total N	Crude HR (95% CI)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
No Diabetes				
Stroke *	197/6621	1.25 (1.02-1.53)	1.29 (1.05-1.59)	1.54 (1.09-2.18)
Coronary heart disease †	174/6173	1.19 (0.96-1.48)	1.20 (0.97-1.48)	1.14 (0.88-1.47)
Composite Events ‡	297/5755	1.23 (1.05-1.64)	1.26 (1.07-1.48)	1.30 (1.03-1.62)
Diabetes				
Stroke *	131/2660	0.95 (0.74-1.22)	0.94 (0.72-1.21)	0.82 (0.56-1.20)
Coronary heart disease †	148/2329	0.96 (0.76-1.22)	0.94 (0.74-1.20)	0.83 (0.61-1.12)
Composite Events ‡	200/2072	0.97 (0.80-1.19)	0.98 (0.80-1.20)	0.82 (0.62-1.09)

Shown are the hazard ratios (HR) (95% confidence interval) per *APOL1* risk variant for stroke, coronary heart disease, and composite events. * Excludes prevalent stroke, and hemorrhagic and non-adjudicated strokes; † Excludes prevalent coronary heart disease; ‡ Excludes prevalent stroke, hemorrhagic strokes, non-adjudicated strokes, and prevalent coronary heart disease.

Model 1 adjusted for age, sex, smoking, hypertension, and lipid medications. [Diabetes, Events/N: Stroke = 129/2620; CHD = 144/2296; Composite = 196/2044; No Diabetes, Events/N: Stroke = 193/6520; CHD = 173/6085; Composite = 294/5675]
Model 2 adjusted for variables in Model 1 plus principal components of ancestry. [Diabetes, Events/N: Stroke = 60/1861; CHD =96/1669; Composite = 113/1489; No Diabetes, Events/N: Stroke = 67/4463; CHD = 125/4225; Composite = 160/3946]

Supplemental Table 6. Association of *APOL1* risk alleles with incident cardiovascular outcomes, stratified by diabetes status, dominant model.

Outcomes	Events/total N	Crude HR (95% CI)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
No Diabetes				
Stroke *	197/6621	1.33 (0.99-1.78)	1.37 (1.02-1.84)	1.40 (0.84-2.35)
Coronary heart disease †	174/6173	1.21 (0.89-1.64)	1.19 (0.88-1.62)	1.13 (0.78-1.62)
Composite Events ‡	297/5755	1.27 (1.00-1.60)	1.28 (1.01-1.62)	1.25 (0.90-1.74)
Diabetes				
Stroke *	131/2660	1.04 (0.73-1.48)	0.99 (0.70-1.42)	0.87 (0.52-1.47)
Coronary heart disease †	148/2329	0.99 (0.71-1.38)	0.99 (0.71-1.39)	0.89 (0.59-1.34)
Composite Events ‡	200/2072	1.08 (0.81-1.44)	1.08 (0.81-1.45)	0.90 (0.62-1.32)

Shown are the hazard ratios (95% confidence interval) for stroke, coronary heart disease, and composite events, comparing individuals with two or one APOL1 risk variants to zero risk variants.
* Excludes prevalent stroke, and hemorrhagic and non-adjudicated strokes; † Excludes prevalent coronary heart disease; ‡ Excludes prevalent stroke, hemorrhagic strokes, non-adjudicated strokes, and prevalent coronary heart disease

Model 1 adjusted for age, sex, smoking, hypertension and lipid medications. [Diabetes, Events/N: Stroke = 129/2620; CHD = 144/2296; Composite = 196/2044; No Diabetes, Events/N: Stroke = 193/6520; CHD = 173/6085; Composite = 294/5675]
Model 2 adjusted for variables in Model 1 plus principal components of ancestry. [Diabetes, Events/N: Stroke = 60/1861; CHD =96/1669; Composite = 113/1489; No Diabetes, Events/N: Stroke = 67/4463; CHD = 125/4225; Composite = 160/3946]

Supplemental Table 7. Association of *APOL1* risk alleles with incident cardiovascular outcomes, stratified by chronic kidney disease (CKD), additive model.

Outcomes	Events/total N	Crude HR (95% CI)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
No CKD				
Stroke *	211/7098	1.16 (0.95-1.41)	1.19 (0.97-1.46)	1.39 (1.02-1.89)
Coronary heart disease †	186/6557	1.03 (0.83-1.28)	1.04 (0.84-1.29)	0.98 (0.75-1.27)
Composite Events ‡	300/6125	1.08 (0.91-1.27)	1.11 (0.94-1.31)	1.11 (0.88-1.39)
CKD				
Stroke *	158/2315	1.04 (0.83-1.30)	1.05 (0.84-1.32)	0.94 (0.66-1.34)
Coronary heart disease †	137/2033	1.12 (0.88-1.41)	1.11 (0.87-1.42)	1.04 (0.77-1.39)
Composite Events ‡	200/1783	1.13 (0.93-1.38)	1.16 (0.94-1.41)	1.07 (0.81-1.40)

Shown are the hazard ratios (HR) (95% confidence interval) per *APOL1* risk variant for stroke, coronary heart disease, and composite events. * Excludes prevalent stroke, and hemorrhagic and non-adjudicated strokes; † Excludes prevalent coronary heart disease; ‡ Excludes prevalent stroke, hemorrhagic strokes, non-adjudicated strokes, and prevalent coronary heart disease. CKD was defined as urine albumin to creatinine ratio ≥ 30 mg/g or eGFR < 60 mL/min/1.73m^2.

Model 1 adjusted for age, sex, smoking, hypertension, lipid medications and diabetes. [CKD, Events/N: Stroke = 152/2228; CHD = 133/1953; Composite = 194/1712; No CKD, Events/N: Stroke = 205/6947; CHD = 184/6428; Composite = 296/6007]
Model 2 adjusted for variables in Model 1 plus principal components of ancestry. [CKD, Events/N: Stroke = 66/1561; CHD = 98/1405; Composite = 112/1230; No CKD, Events/N: Stroke = 86/4788; CHD = 123/4489; Composite = 161/4205]

Supplemental Table 8. Association of *APOL1* risk alleles with incident cardiovascular outcomes, stratified by chronic kidney disease (CKD), dominant model.

Outcomes	Events/total N	Crude HR (95% CI)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
No CKD				
Stroke *	211/7098	1.19 (0.90-1.57)	1.21 (0.91-1.60)	1.38 (0.88-2.16)
Coronary heart disease †	186/6557	0.99 (0.74-1.32)	0.99 (0.74-1.33)	0.97 (0.68-1.39)
Composite Events ‡	300/6125	1.07 (0.85-1.35)	1.11 (0.87-1.40)	1.11 (0.80-1.53)
CKD				
Stroke *	158/2315	1.23 (0.89-1.71)	1.22 (0.88-1.71)	1.01 (0.61-1.66)
Coronary heart disease †	137/2033	1.29 (0.91-1.84)	1.25 (0.87-1.78)	1.08 (0.72-1.63)
Composite Events ‡	200/1783	1.34 (1.00-1.79)	1.32 (0.98-1.78)	1.09 (0.74-1.61)

Shown are the hazard ratios (HR) (95% confidence interval) for stroke, coronary heart disease, and composite events, comparing individuals with 1 or 2 risk variants to those with 0 risk variants. * Excludes prevalent stroke, and hemorrhagic and non-adjudicated strokes; † Excludes prevalent coronary heart disease; ‡ Excludes prevalent stroke, hemorrhagic strokes, non-adjudicated strokes, and prevalent coronary heart disease. CKD was defined as urine albumin to creatinine ratio ≥ 30 mg/g or eGFR < 60 mL/min/1.73m^2.

Model 1 adjusted for age, sex, smoking, hypertension, lipid medications and diabetes. [CKD, Events/N: Stroke = 152/2228; CHD = 133/1953; Composite = 194/1712; No CKD, Events/N: Stroke = 205/6947; CHD = 184/6428; Composite = 296/6007]
Model 2 adjusted for variables in Model 1 plus proportion African ancestry [CKD, Events/N: Stroke = 66/1561; CHD = 98/1405; Composite = 112/1230; No CKD, Events/N: Stroke = 86/4788; CHD = 123/4489; Composite = 161/4205].

Supplemental Table 9. Association of *APOL1* risk alleles with incident cardiovascular outcomes, stratified by estimated glomerular filtration rate < vs. ≥ 60 ml/min/1.73m^2, recessive model.

Outcomes	Events/total	Crude HR (95% CI)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
eGFR < 60				
Stroke *	55/1090(5.0%)	1.19 (0.60-2.37)	1.20 (0.58-2.48)	0.76 (0.21-2.64)
Coronary heart disease †	67/947(7.1%)	0.95 (0.49-1.87)	0.84 (0.40-1.78)	0.60 (0.21-1.68)
Composite Events ‡	83/803(10.3%)	1.16 (0.66-2.04)	1.10 (0.60-2.00)	0.96 (0.42-2.18)
eGFR ≥ 60				
Stroke *	276/8285(3.3%)	1.05 (0.74-1.48)	1.12 (0.79-1.60)	1.50 (0.91-2.49)
Coronary heart disease †	256/7643(3.3%)	1.14 (0.80-1.62)	1.16 (0.82-1.66)	1.08 (0.70-1.67)
Composite Events ‡	417/7105(5.9%)	1.10 (0.84-1.46)	1.16 (0.87-1.53)	1.19 (0.82-1.72)

Abbreviations: eGFR, estimated glomerular filtration rate
Shown are the hazard ratios (HR) (95% confidence interval) for stroke, coronary heart disease, and composite events, comparing individuals with 2 risk variants to those with 0 or 1. * Excludes prevalent stroke, and hemorrhagic and non-adjudicated strokes; † Excludes prevalent coronary heart disease; ‡ Excludes prevalent stroke, hemorrhagic strokes, non-adjudicated strokes, and prevalent coronary heart disease.

Model 1 adjusted for age, sex, smoking, hypertension, lipid medications and diabetes.
[eGFR≥60, Events/N: Stroke =271/8110 ; CHD = 253/7492; Composite = 411/6967; eGFR <60, Events/N: Stroke = 36/572; CHD = 64/889; Composite = 79/752]

Model 2 adjusted for variables in Model 1 plus principal components of ancestry.
[eGFR≥60, Events/N: Stroke = 105/5599; CHD = 176/5245; Composite = 227/4892; eGFR<60, Events/N: Stroke = 22/725; CHD = 45/649; Composite = 46/543].

Supplemental Table 10. Association of *APOL1* risk alleles with incident cardiovascular outcomes, stratified by urine albumin to creatinine ratio (ACR) ≥ vs. < 30 mg/g, recessive model.

Outcomes	Events/total	Crude	Model 1	Model 2
		HR (95% CI)	HR (95% CI)	HR (95% CI)
ACR ≥ 30 mg/g				
Stroke *	127/1653	0.68(0.40-1.17)	0.72(0.41-1.26)	0.64(0.25-1.62)
Coronary heart disease †	105/1447	0.86(0.50-1.49)	0.99(0.57-1.72)	0.91(0.46-1.79)
Composite Events ‡	160/1282	0.85(0.55-1.33)	0.95(0.60-1.49)	0.91(0.49-1.73)
ACR < 30 mg/g				
Stroke *	204/7722	1.29(0.88-1.89)	1.39(0.95-2.04)	1.90(1.10-3.28)
Coronary heart disease †	218/7143	1.18(0.81-1.73)	1.14(0.77-1.68)	0.97(0.59-1.60)
Composite Events ‡	340/6626	1.22(0.90-1.64)	1.25(0.92-1.69)	1.25(0.84-1.87)

Abbreviations: ACR, urine albumin to creatinine ratio
Shown are the hazard ratios (HR) (95% confidence interval) for stroke, coronary heart disease, and composite events, comparing individuals with 2 risk variants to those with 0 or 1. * Excludes prevalent stroke, and hemorrhagic and non-adjudicated strokes; † Excludes prevalent coronary heart disease; ‡ Excludes prevalent stroke, hemorrhagic strokes, non-adjudicated strokes, and prevalent coronary heart disease.

Model 1 adjusted for age, sex, smoking, hypertension, lipid medications and diabetes.
[ACR≥30, Events/N: Stroke =123/1611 ; CHD = 104/1412; Composite = 157/1252; ACR <30, Events/N: Stroke = 80/5257; CHD = 213/6969; Composite = 333/6467]

Model 2 adjusted for variables in Model 1 plus principal components of ancestry.
[ACR≥30, Events/N: Stroke = 48/1118; CHD = 77/1004; Composite = 89/892; ACR<30, Events/N: Stroke = 79/5206; CHD = 144/4890; Composite = 184/4543]

Supplemental Table 11. Association of *APOL1* risk alleles with incident cardiovascular outcomes, stratified by age (<65 or ≥ 65 years), recessive model.

Outcomes	Events/total N	Crude HR (95% CI)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
Age ≥ 65 years				
Stroke *	214/4188	1.23 (0.84-1.80)	1.27 (0.86-1.87)	1.55 (0.86-2.78)
Coronary heart disease †	189/3753	1.35 (0.92-1.98)	1.32 (0.88-1.96)	1.16 (0.72-1.87)
Composite Events ‡	304/3373	1.38 (1.02-1.87)	1.40 (1.03-1.91)	1.36 (0.90-2.05)
Age < 65 years				
Stroke *	117/5187	0.95 (0.55-1.63)	0.91 (0.53-1.57)	1.02 (0.47-2.19)
Coronary heart disease †	134/4837	0.84 (0.49-1.44)	0.85 (0.50-1.46)	0.71 (0.34-1.48)
Composite Events ‡	196/4535	0.88 (0.57-1.36)	0.87 (0.56-1.35)	0.90 (0.50-1.63)

Shown are the hazard ratios (HR) (95% confidence interval) for stroke, coronary heart disease, and composite events, comparing individuals with 2 *APOL1* risk variants to those with 0 or 1. *Excludes prevalent stroke, and hemorrhagic and non-adjudicated strokes; † Excludes prevalent coronary heart disease; ‡ Excludes prevalent stroke, hemorrhagic strokes, non-adjudicated strokes, and prevalent coronary heart disease.

Model 1 adjusted for sex, smoking, hypertension, lipid medications, and diabetes. [Age ≥ 65 years, Events/N: Stroke = 207/4124; CHD = 184/3665; Composite = 295/3298; Age < 65 years, Events/N: Stroke = 117/5104; CHD = 133/4716; Composite = 195/4421]
Model 2 adjusted for variables in Model 1 plus ancestry principal components. [Age ≥ 65 years, Events/N: Stroke = 78/2906; CHD = 136/2652; Composite = 168/2396; Age < 65 years, Events/N: Stroke = 49/3418; CHD = 85/3242; Composite = 105/3039]
$P_{interaction}$ for age (< vs. ≥ 65 years) on the association of *APOL1* high-risk genotypes with incident stroke, CHD and the composite outcome in the fully adjusted model (model 2) were 0.67, 0.23, and 0.35, respectively

Supplemental Table 12. Association of *APOL1* risk alleles with incident cardiovascular outcomes, stratified by age, additive model.

Outcomes	Events/total N	Crude HR (95% CI)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
Age ≥ 65 years				
Stroke *	214/4188	1.24 (1.02-1.51)	1.25 (1.03-1.52)	1.17 (0.84-1.62)
Coronary heart disease †	189/3753	1.16 (0.94-1.42)	1.14 (0.92-1.40)	1.12 (0.87-1.43)
Composite Events ‡	304/3373	1.26 (1.07-1.48)	1.27 (1.08-1.50)	1.21 (0.97-1.52)
Age < 65 years				
Stroke *	117/5187	0.96 (0.73-1.25)	0.95 (0.73-1.24)	1.16 (0.73-1.72)
Coronary heart disease †	134/4837	1.01 (0.79-1.29)	0.99 (0.77-1.28)	0.82 (0.59-1.14)
Composite Events ‡	196/4535	0.97 (0.79-1.19)	0.95 (0.77-1.17)	0.91 (0.68-1.21)

Legend. Shown are the hazard ratios (HR) (95% confidence interval) per *APOL1* risk variant for stroke, coronary heart disease, and composite events. * Excludes prevalent stroke, and hemorrhagic and non-adjudicated strokes; † Excludes prevalent coronary heart disease; ‡ Excludes prevalent stroke, hemorrhagic strokes, non-adjudicated strokes, and prevalent coronary heart disease.

Model 1 adjusted for sex, smoking, hypertension, lipid medications, and diabetes. [Age ≥ 65 years, Events/N: Stroke = 207/4124; CHD = 184/3665; Composite = 295/3298; Age < 65 years, Events/N: Stroke = 117/5104; CHD = 133/4716; Composite = 195/4421]
Model 2 adjusted for variables in Model 1 plus plus principal components of ancestry. [Age ≥ 65 years, Events/N: Stroke = 78/2906; CHD = 136/2652; Composite = 168/2396; Age < 65 years, Events/N: Stroke = 49/3418; CHD = 85/3242; Composite = 105/3039]

Supplemental Table 13. Association of *APOL1* risk alleles with incident cardiovascular outcomes, stratified by age, dominant model.

Outcomes	Events/total N	Crude HR (95% CI)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
Age ≥ 65 years				
Stroke*	214/4188	1.39 (1.05-1.85)	1.41 (1.06-1.89)	1.07 (0.67-1.70)
Coronary heart disease †	189/3753	1.14 (0.85-1.53)	1.12 (0.83-1.50)	1.16 (0.82-1.65)
Composite Events ‡	304/3373	1.33 (1.05-1.68)	1.35 (1.06-1.72)	1.25 (0.91-1.72)
Age < 65 years				
Stroke*	117/5187	0.95 (0.66-1.36)	0.91 (0.63-1.32)	1.31 (0.72-2.38)
Coronary heart disease †	134/4837	1.09 (0.77-1.55)	1.06 (0.75-1.51)	0.89 (0.52-1.25)
Composite Events ‡	196/4535	1.00 (0.75-1.33)	0.97 (0.73-1.29)	0.87 (0.59-1.29)

Shown are the hazard ratios (HR) (95% confidence interval) for stroke, coronary heart disease, and composite events, comparing individuals with 1 or 2 risk variants to those with 0 risk variants. * Excludes prevalent stroke, and hemorrhagic and non-adjudicated strokes; † Excludes prevalent coronary heart disease; ‡ Excludes prevalent stroke, hemorrhagic strokes, non-adjudicated strokes, and prevalent coronary heart disease..

Model 1 adjusted for sex, smoking, hypertension, lipid medications, and diabetes. [Age ≥ 65 years, Events/N: Stroke = 207/4124; CHD = 184/3665; Composite = 295/3298; Age < 65 years, Events/N: Stroke = 117/5104; CHD = 133/4716; Composite = 195/4421]
Model 2 adjusted for variables in Model 1 plus principal components of ancestry. [Age ≥ 65 years, Events/N: Stroke = 78/2906; CHD = 136/2652; Composite = 168/2396; Age < 65 years, Events/N: Stroke = 49/3418; CHD = 85/3242; Composite = 105/3039]

Supplemental Table 14. Association of *APOL1* risk alleles with incident cardiovascular outcomes, stratified by sickle cell trait, recessive model.

Outcomes	Events/total	Crude HR (95% CI)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
Presence of sickle cell trait				
Stroke *	----	----	----	-----
Coronary heart disease †	27/627	1.58 (0.60-4.18)	1.60 (0.60-4.26)	0.96 (0.26-3.61)
Composite Events ‡	36/589	1.11(0.43-2.86)	1.19 (0.46-3.09)	0.96 (0.25-3.65)
Absence of sickle cell trait				
Stroke *	308/8578	1.15 (0.84-1.58)	1.21 (0.88-1.67)	1.02 (0.47-2.19)
Coronary heart disease †	291/7867	1.07 (0.77-1.50)	1.05 (0.74-1.48)	0.97 (0.63-1.47)
Composite Events ‡	455/7238	1.12 (0.87-1.46)	1.13 (0.87-1.48)	1.15 (0.81-1.63)

Shown are the hazard ratios (HR) (95% confidence interval) for stroke, coronary heart disease, and composite events, comparing individuals with 2 risk variants to those with 0 or 1. * Excludes prevalent stroke, and hemorrhagic and non-adjudicated strokes; † Excludes prevalent coronary heart disease; ‡ Excludes prevalent stroke, hemorrhagic strokes, non-adjudicated strokes, and prevalent coronary heart disease.

Model 1 adjusted for age, sex, smoking, hypertension, lipid medications and diabetes. [Presence of sickle cell trait : Events/N: Stroke = 19/681; CHD = 27/613; Composite = 36/572; Absence of sickle cell trait, Events/N: Stroke = 299/8362; CHD = 285/7674; Composite = 445/7064]
Model 2 adjusted for variables in Model 1 plus plus principal components of ancestry. [Presence of sickle cell trait: Events/N: Stroke = 5/459; CHD = 20/418; Composite = 19/388; Absence of sickle cell trait: Events/N: Stroke = 121/5800; CHD = 201/5465; Composite = 251/4990]
$P_{interaction}$ for sickle cell trait on the association of *APOL1* high-risk genotypes with incident stroke, CHD and the composite outcome in the fully adjusted model (model 2) were 0.97, 0.52, and 0.77, respectively.

Supplemental Table 15. Hazard ratios (95% confidence interval) of *APOL1* risk alleles with incident stroke subtypes comparing 2 vs 0 or 1 *APOL1* risk variants.

Stroke Subtypes	Events/Total	Crude HR (95% CI)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
Overall				
Cardioembolic	56/9100	1.30 (0.64-2.65)	1.30 (0.61-2.76)	1.38 (0.46-4.11)
Large vessel disease	36/9080	0.40 (0.10-1.66)	0.39 (0.09-1.62)	---
Small vessel disease	64/9108	1.72 (0.94-3.17)	1.85 (1.004-3.42)	3.74 (1.61-8.65)
Unknown	159/9203	0.98(0.61-1.56)	1.06(0.66-1.70)	1.01(0.48-2.14)
Diabetes				
Cardioembolic	27/2556	0.50(0.12-2.07)	0.51(0.12-2.15)	0.44(0.06-3.47)
Large vessel disease	15/2544	------	------	-----
Small vessel disease	25/2554	1.91 (0.76-4.79)	2.03 (0.80-5.12)	2.67(0.79-9.04)
Unknown	57/2586	0.59 (0.24,1.48)	0.62 (0.25,1.56)	-----
No Diabetes				
Cardioembolic	29/6453	2.24(0.96-5.24)	2.48 (1.00-6.18)	3.50(0.84-14.52)
Large vessel disease	21/6445	0.69(0.17-3.19)	0.78(0.18-3.37)	-----
Small vessel disease	39/6463	1.54(0.68-3.49)	1.72 (0.76-3.92)	5.10(1.55-16.56)
Unknown	99/6523	1.26(0.73-2.18)	1.38(0.80-2.40)	1.85(0.83-4.09)

‡ Excludes prevalent stroke, hemorrhagic strokes and non-adjudicated stroke.

Model 1 adjusted for age, sex, smoking, hypertension, lipid medications and diabetes (except for analysis stratified by diabetes status).
Model 2 adjusted for variables in Model 1 plus plus principal components of ancestry

Supplemental Table 16. Association of *APOL1* risk alleles with serum lipids, recessive model.

	Model 1	***P-value***	**Model 2**	***P-value***
Total cholesterol	2.91 (1.18)	0.01	0.99 (1.41)	0.48
High-density lipoprotein cholesterol	0.99 (0.45)	0.03	0.46 (0.54)	0.39
Low-density lipoprotein cholesterol	1.91 (1.06)	0.07	0.53 (1.27)	0.68
Triglycerides	0.46 (2.17)	0.83	0.30 (2.38)	0.90

Shown are the R^2 values for the linear regression model for serum lipid values, using a recessive model (2 vs 1 or 0 *APOL1* risk variants).

Model 1 adjusted for age, sex, smoking, hypertension, lipid medications, and diabetes. [HDL (N) =10,247; LDL (N) = 10,172; TG (N) =10,315, TC (N) =10,320]

Model 2 adjusted for variables in Model 1 plus principal components of ancestry. [HDL (N) = 6,917; LDL (N) = 6,864; TG (N) = 6,965; TC (N) =6,968]

Supplemental Table 17. Association of *APOL1* risk alleles with serum lipids, additive model.

	Model 1	***P-value***	**Model 2**	***P-value***
Total cholesterol	1.985(0.58)	0.01	0.88 (0.70)	0.21
High-density lipoprotein cholesterol	0.64(0.22)	0.003	0.42(0.27)	0.11
Low-density lipoprotein cholesterol	1.51(0.52)	0.004	0.61(0.63)	0.33
Triglycerides	-1.41(1.07)	0.18	-0.88(1.18)	0.46

Shown are the R^2 values for the linear regression model for serum lipid values, using a recessive model (2 vs 1 or 0 *APOL1* risk variants).

Model 1 adjusted for age, sex, smoking, hypertension, lipid medications, and diabetes. [HDL (N) =10,247; LDL (N) = 10,172; TG (N) =10,315, TC (N) =10,320]

Model 2 adjusted for variables in Model 1 plus principal components of ancestry. [HDL (N) = 6,917; LDL (N) = 6,864; TG (N) = 6,965; TC (N) =6,968]

Supplemental Table 18. Association of *APOL1* risk alleles with serum lipids, dominant model.

	Model 1	***P-value***	**Model 2**	***P-value***
High-density lipoprotein cholesterol	0.77 (0.30)	0.01	0.59(0.37)	0.11
Low-density lipoprotein cholesterol	1.98 (0.72)	0.006	0.92(0.88)	0.29
Triglycerides	-2.88(1.47)	0.049	-1.82(1.63)	0.26
Total cholesterol	2.41(0.80)	0.003	1.21(0.99)	0.21

Shown are the R^2 values for the linear regression model or serum lipid values, using a dominant model (2 vs 1 or 0 *APOL1* risk variants).

Model 1 adjusted for age, sex, smoking, hypertension, lipid medications, and diabetes. [HDL (N) =10,247; LDL (N) = 10,172; TG (N) =10,315, TC (N) =10,320]
Model 2 adjusted for variables in Model 1 plus principal components of ancestry. [HDL (N) = 6,917; LDL (N) = 6,864; TG (N) = 6,965;TC (N) =6,968]