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ORIGINAL RESEARCH

Association Between *APOL1* Genotypes and Risk of Cardiovascular Disease in MESA (Multi-Ethnic Study of Atherosclerosis)

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Background*—APOL1* genetic variants confer an increased risk for kidney disease. Their associations with cardiovascular disease (CVD) are less certain. We aimed to compare the prevalence of subclinical CVD and incidence of atherosclerotic CVD and heart failure by *APOL1* genotypes among self-identified black participants of MESA (Multi-Ethnic Study of Atherosclerosis).

***Methods and Results*—**Cross-sectional associations of *APOL1* genotypes (high-risk=2 alleles; low-risk=0 or 1 allele) with coronary artery calcification, carotid-intimal media thickness, and left ventricular mass were evaluated using logistic and linear regression. Longitudinal associations of *APOL1* genotypes with incident myocardial infarction, stroke, coronary heart disease, and congestive heart failure were examined using Cox regression. We adjusted for African ancestry, age, and sex. We also evaluated whether hypertension or kidney function markers explained the observed associations. Among 1746 participants with *APOL1* genotyping (mean age 62 years, 55% women, mean cystatin C–based estimated glomerular filtration rate 89 mL/min per 1.73 m^2, 12% with albuminuria), 12% had the high-risk genotypes. We found no difference in prevalence or severity of coronary artery calcification, carotid-intimal media thickness, or left ventricular mass by *APOL1* genotypes. The *APOL1* high-risk group was 82% more likely to develop incident heart failure compared with the low-risk group (95% confidence interval, 1.01–3.28). Adjusting for hypertension (hazard ratio, 1.80; 95% confidence interval, 1.00–3.24) but not markers of kidney function (hazard ratio, 1.86; 95% confidence interval, 1.03–3.35) slightly attenuated this association. The *APOL1* high-risk genotypes were not significantly associated with other clinical CVD outcomes.

***Conclusions*—**Among blacks without baseline CVD, the *APOL1* high-risk variants may be associated with increased risk for incident heart failure but not subclinical CVD or incident clinical atherosclerotic CVD. **(*J Am Heart Assoc*. 2017;6:e007199. DOI: 10.1161/JAHA.117.007199.)**

Key Words: *APOL1* • cardiovascular disease • coronary artery calcium • heart failure • Multi-Ethnic Study of Atherosclerosis

Black people are significantly more likely to develop end-stage renal disease compared with other races, even after accounting for traditional risk factors for kidney disease.[1–3] This excess burden of progressive kidney disease has been attributed, in part, to the higher prevalence of variants in the gene encoding for apolipoprotein L1 (*APOL1*), located on chromosome 22, among self-identified blacks.[4–9] The *APOL1* risk variants (G1 and G2), which confer protection against African sleeping sickness,[4,5,10] are associated with increased risk for various types of kidney disease, including focal segmental glomerulosclerosis,[11] HIV-associated nephropathy,[11,12] lupus nephritis,[13] hypertension-attributed chronic kidney disease,[6] and possibly accelerated progression of diabetic kidney disease.[7]

While significant advances have been made in our understanding of *APOL1*-associated kidney disease,[14] the specific mechanisms by which these genetic variants cause kidney disease remain uncertain. Because of the elevated risks for

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Accompanying Tables S1 through S3 are available at http://jaha.ahajournals.org/content/6/12/e007199/DC1/embed/inline-supplementary-material-1.pdf

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Clinical Perspective

What Is New?

- *APOL1* high-risk variants may be associated with an increased risk for incident heart failure but not subclinical or incident clinical atherosclerotic disease.

What Are the Clinical Implications?

- Further clarification on the role of *APOL1* high-risk variants in heart failure is necessary to optimize the care of individuals with this high-risk genetic background.

cardiovascular disease (CVD) and heart failure (HF) among people with chronic kidney disease compared with the general population,[15] investigation of the cardiovascular risk of individuals who carry the *APOL1* high-risk variants is an issue of active study. Apolipoprotein L1 (Apol1) has been localized to endothelial and vascular smooth muscle cells within the kidney[16,17] and is also known to circulate in plasma with high-density lipoprotein particles,[18,19] suggesting a potential role of the *APOL1* risk variants in CVD. Studies to date on this topic, however, have been conflicting. In a cross-sectional analysis of SPRINT (Systolic Blood Pressure Intervention Trial), an increasing number of *APOL1* risk variants was not associated with prevalent clinical atherosclerotic CVD.[20] In longitudinal analyses of community-based blacks, postmenopausal women, or older individuals, *APOL1* risk variants have been associated with increased cardiovascular morbidity.[21,22] Other longitudinal analyses in the general population and among blacks with diabetes mellitus or hypertension, however, have reported either no association with clinical CVD or even reduced risk for subclinical CVD.[23–25]

Racial disparities in the incidence of CVD are well described.[26–29] In particular, the incidence of HF is much higher among blacks compared with whites.[26,27,29] Chronic kidney disease has also been associated with an increased risk of incident HF, especially among blacks.[15] Whether *APOL1* risk variants partially explain the excess burden of HF in blacks is not known.

In the present study, we aimed to determine whether the *APOL1* high-risk variants are associated with subclinical CVD, incident clinical atherosclerotic CVD, and incident HF among self-identified black participants of MESA (Multi-Ethnic Study of Atherosclerosis).

Methods

The data, analytic methods, and study materials will not be made available to other researchers for purposes of reproducing the results or replicating the procedure.

Study Population

MESA was a population-based cohort study designed to investigate the pathogenesis of CVD, with an emphasis on subclinical CVD progression. Details regarding the study design of MESA have previously been published.[30] Briefly, 6814 men and women of 4 racial/ethnic groups (white, black, Hispanic, and Asian), aged 45 to 84 years, without baseline clinical CVD or HF were recruited from 6 communities in the United States (Baltimore, Maryland; Chicago, Illinois; Forsyth County, North Carolina; Los Angeles County, California; New York, New York; and St. Paul, Minnesota). Enrollment for the first examination occurred between July 2000 and August 2002, followed by 4 more examinations: examination 2 (from September 2002 to February 2004), examination 3 (from March 2004 to September 2005), examination 4 (from September 2005 to May 2007), and examination 5 (from April 2010 to December 2011).[30–32] Each participating MESA site obtained approval from their institutional review board and all participants provided informed consent.[32] Among the 6814 participants, a total of 3217 (1746 black and 1471 Hispanic) were successfully genotyped for the *APOL1* risk variants. The *APOL1* risk variants are extremely rare in people of European descent.[33,34] We excluded individuals of Hispanic origin because of the relatively low prevalence of *APOL1* risk variants in this ethnic group and the potential for confounding by ethnicity.[35] Thus, our study population consisted of the 1746 self-identified black participants with available *APOL1* genotyping.

Measurement of Outcomes

Measurements of subclinical CVD (coronary artery calcification [CAC], carotid-intimal media thickness [CIMT], and left ventricular mass) were obtained at baseline. Chest computed tomography by either cardiac-gated electron-beam computed tomography scanner (Chicago, Los Angeles, and New York) or multidetector computed tomography (Baltimore, Forsyth County, and St. Paul) was used to measure CAC. All scans were read centrally by method of Agatston, with the average of 2 consecutive scans used to determine baseline CAC score.[30,31,36] We defined presence of CAC as having an Agatston score greater than zero. CIMT was defined as the mean of the maximum intimal-media thickness of the near and far walls of the common carotid arteries, which were measured by high-resolution B-mode ultrasound.[30,31,37] Cardiac magnetic resonance imaging was performed at the baseline visit using scanners with 1.5-T magnets, a 4-element, phased-array surface coil placed anteriorly and posteriorly, ECG gating, and brachial artery blood pressure (BP) monitoring.[30] All images were read centrally and left ventricular mass determined as previously described.[30,38]

Study participants were followed for clinical atherosclerotic CVD and HF outcomes by telephone interviews every 9 to 12 months.[39] All self-reported diagnoses were confirmed by review of death certificates and medical records. Interviews with physicians, next-of-kin, and friends were also conducted to verify out-of-hospital deaths.[30,39] Using predefined criteria, each event was adjudicated by 2 independent reviewers. We utilized a composite outcome of incident coronary heart disease (CHD), which included myocardial infarction (MI), definite or probable angina (if followed by revascularization), resuscitated cardiovascular arrest, and CHD death. A diagnosis of MI was made based on a combination of symptoms, ECG abnormalities, and changes in cardiac biomarker levels. Angina was characterized by symptoms of ischemia plus either physician diagnosis and treatment of angina or objective imaging to support reversible myocardial ischemia or obstructive coronary artery disease. CHD death was specified by the occurrence of an MI within 28 days of death, chest pain within 72 hours of death, or history of CHD without a known noncardiac or nonatherosclerotic cause of death. Stroke was defined as a neurologic deficit lasting for more than 24 hours (or until death) with a clinically relevant brain lesion on imaging.[39,40] A diagnosis of congestive HF required clinical signs or symptoms of congestive HF that were further supported by physician diagnosis and treatment or imaging (chest x-ray, echocardiogram, or ventriculography).[40]

Genotyping

The *APOL1* risk variants (rs73885319 and rs71785313) were genotyped using TaqMan assays (Applied Biosystems 7900) and DNA samples (extracted from buffy coat) collected at the baseline examination.[4,5,30] Consistent with prior studies, G1 (risk allele for rs73885319 and rs60910145) is comprised of 2 missense mutations (S342G and I384M) and G2 (risk allele for rs71785313) is characterized by a 6 base pair deletion (del N388/Y389).[4,5] We used a recessive genetic model, defining the *APOL1* high-risk genotypes as having 2 risk alleles (G1/G1, G1/G2, or G2/G2) and the low-risk genotypes as having 1 or no risk alleles (G1/G0, G2/G0, G0/G0). Global African ancestry proportion was estimated using 406 ancestry informative markers from the Affymetrix 6.0 array, and 4 ancestral populations in ADMIXMAP software. The ancestry estimation was performed using 8227 European American, black, Hispanic, and Chinese MESA and MESA Family participants.

Covariates

Details on demographic data, personal and family medical histories, socioeconomic status, and medications were obtained using questionnaires.[30] Physical activity was ascertained using the MESA Typical Week Physical Activity Survey, which was adapted from the Cross-Cultural Activity Participation Survey.[30,41] At each visit, 3 resting BPs were measured by trained personnel using a Dinamap model Pro 100 automated oscillometric sphygmomanometer (Critikon) and the average of the last 2 measurements was used.[30,41–43] Hypertension was defined as a systolic BP $\geq$140 mm Hg, diastolic BP $\geq$90 mm Hg, or use of antihypertensive medications.[44] Measurements for lipid parameters were performed at a central laboratory using blood that had been collected after a 12-hour fast. Total and high-density lipoprotein cholesterol levels were measured using the cholesterol oxidase method (Roche Diagnostics), triglycerides were measured using the triglyceride GB reagent (Roche Diagnostics), and low-density lipoprotein cholesterol was estimated using the Friedewald equation (if triglycerides were $<$400 mg/dL).[42,43,45] Serum glucose was also measured centrally from a fasting sample using the Vitros analyzer (Johnson & Johnson Clinical Diagnostics, Inc).[42] Diabetes mellitus was defined as a fasting glucose $\geq$126 mg/dL or use of glucose-lowering medications.[46]

Kidney function was assessed by measurement of cystatin C from frozen serum specimens that had been stored at $-$70°C. As previously described, cystatin C was measured by a particle-enhanced immunonephelometric assay (N Latex Cystatin C, Siemens) on a nephelometer (BNII, Siemens) and corrected for assay drift.[47,48] Glomerular filtration was estimated using the Chronic Kidney Disease-Epidemiology Collaboration equation.[49] Albuminuria, which we defined as a urine albumin to creatinine ratio (UACR) $\geq$30 mg/g, was measured from a single morning urine sample. Urinary albumin was determined by nephelometry using the Array 360 CE Protein Analyzer (Beckman Instruments Inc), whereas urinary creatinine was determined by the rate Jaffe method using the Vitros 950IRC instrument (Johnson & Johnson Clinical Diagnostics, Inc).[47,50] Glomerular filtration rate (GFR) was estimated at examinations 1, 3, 4, and 5, whereas UACR was measured at examinations 1, 2, 3, and 5.

Statistical Analyses

Baseline demographic, socioeconomic, and clinical characteristics were compared by *APOL1* risk status (high versus low risk) using means (SD), medians (interquartile range), or counts (percentage). The cross-sectional associations between *APOL1* risk status and each measure of subclinical CVD were then evaluated using linear or logistic regression. CAC was assessed in 2 ways, by presence and by severity. First, we categorized CAC as being either present (Agatston score $>$0) or absent. Since the prevalence of CAC was high (43% in our study population), the rare disease assumption required for the odds ratio to be approximately equal to the

relative risk (RR) is not met and odds ratios are likely to overestimate RR. For each of the *i* participants, i=1, . . ., n, let p_i be the probability that the participant has a positive Agatston score (CAC >0) and $\mathbf{x}_i$ be a vector of predictors; the logistic regression postulates $p_i = \exp(\mathbf{x}_i^T\boldsymbol{\beta})/(1+\exp(\mathbf{x}_i^T\boldsymbol{\beta}))$. The parameters $\boldsymbol{\beta}$, when exponentiated, are interpreted as odds ratios. To avoid the frequent misinterpretation of the odds ratio as an estimate of the RR, we modeled the RR directly by expressing $p_i = \exp(\mathbf{x}_i^T\boldsymbol{\beta})$. The parameters $\boldsymbol{\beta}$, when exponentiated, are interpreted as RRs, this is the RR regression model. In this model, the error distribution is binomial conditional on the vector of covariates, $\mathbf{x}$. Thus, the efficient model for estimating the coefficients is the model that uses what is termed the log link with binomial error.[51–53] Second, among participants with detectable CAC, we examined the association between *APOL1* risk status and severity of calcification [ln(Agatston score)] using linear regression models. CIMT and left ventricular mass were assessed as continuous variables, also using linear regression models. For these analyses, we constructed a series of models: (1) model 1: unadjusted model; (2) model 2: adjusted for age, sex, and African ancestry; (3) model 3a: model 2+baseline hypertension; (4) model 3b: model 2+time-updated cystatin C–based estimated GFR and log-transformed UACR; and (5) model 3c: model 2+baseline hypertension+time-updated cystatin C–based estimated GFR and log-transformed UACR. Given that genotypes are unlikely to be confounded by clinical conditions that develop later in life, we treated model 2 as our final model. Furthermore, models 3a–c were intended to assess for mediation by BP and/or kidney disease, so these models were only utilized when a main effect was observed. We then used Cox proportional hazards models to study the longitudinal association between *APOL1* risk status and incident MI, stroke, CHD, and HF. A similar set of nested models was applied to these analyses. The proportional hazards assumption was checked using Schoenfeld residuals. In sensitivity analyses, we repeated the above analyses using additive and dominant genetic models. No adjustments were made for multiple comparisons, as our a priori hypothesis focused on these specific outcomes.[54] Statistical analyses were performed using SPSS version 24 (IBM Corp). *P* values <0.05 were considered to be statistically significant.

Results

Baseline Characteristics

Among 6814 MESA participants, 1746 were black and had *APOL1* genotyping available. Of these 1746 individuals included in our study population, 762 (44%), 771 (44%), and 213 (12%) had 0, 1, and 2 *APOL1* risk alleles, respectively. Therefore, when considering a recessive genetic model, 213 (12%) had the *APOL1* high-risk genotypes (2 risk alleles), and 1533 (88%) had the low-risk genotypes (0 or 1 risk allele). At baseline, the mean age was 62 years, 55% were women, mean cystatin C–based estimated GFR was 89 mL/min per 1.73 m^2, and 12% had albuminuria (defined as a UACR ≥30 mg/g). Compared with the *APOL1* high-risk group, more individuals in the low-risk group had a family history of heart disease. Otherwise, the 2 *APOL1* risk groups were similar at baseline (Table 1).

Coronary Artery Calcium

At baseline, 758 (43%; 77 *APOL1* high-risk and 681 *APOL1* low-risk) individuals had evidence of CAC. In unadjusted analyses, individuals with the *APOL1* high-risk genotypes were 19% less likely to have a CAC score >0 compared with those with the low-risk genotypes (model 1: RR, 0.81; 95% confidence interval [CI], 0.67–0.98). This difference, however, was no longer statistically significant once African ancestry, age, and sex were added to the model (model 2: adjusted RR, 0.88; 95% CI, 0.75–1.03). Among individuals with detectable CAC, the *APOL1* high-risk genotypes were not significantly associated with CAC severity (model 2: adjusted relative difference, 1.41; 95% CI, 0.93–2.16) (Table 2). Similar conclusions were obtained when using an additive or dominant genetic model (Table S1).

Common CIMT

Baseline common CIMT, another measure of subclinical atherosclerosis, was similar between *APOL1* high- and low-risk individuals (mean 0.92 and 0.90 mm, respectively). In unadjusted analyses and analyses adjusted for African ancestry, age, and sex, there was no significant difference in severity of CIMT by *APOL1* risk status (Table 2). The use of an additive or dominant genetic model yielded similar results (Table S1).

Left Ventricular Mass

Left ventricular mass was similar between *APOL1* high- and low-risk individuals at baseline (mean 162 g versus 158 g, respectively). When considering recessive, additive, and dominant genetic models, the *APOL1* high-risk variants were not significantly associated with left ventricular mass (Table 2; Table S1).

Incident CVD Events

Over a mean follow-up of 11.4 years, 125 (7%) individuals developed the composite outcome of incident CHD. Incident MI and stroke occurred in 47 (3%) and 65 (4%) individuals,

Table 1. Baseline Characteristics of Study Population, by *APOL1* Risk Status

Characteristic	*APOL1* Low-Risk (n=1533)	*APOL1* High-Risk (n=213)
Age, y	62±10	62±10
Women	847 (55)	105 (49)
Education		
Less than high school	171 (11)	32 (15)
High school graduate	304 (20)	38 (18)
Post-secondary education	1045 (69)	142 (67)
Employment		
Employed	610 (40)	83 (39)
Unemployed/employed part-time	114 (8)	22 (10%)
Retired/homemaker	795 (52)	107 (51)
Annual family income		
<$25 000	429 (30)	59 (30)
$25 000 to $49 999	462 (33)	61 (31)
$50 000 to $74 999	276 (20)	39 (20)
≥$75 000	247 (18)	35 (18)
Smoking status		
Never	692 (46)	99 (47)
Former	545 (36)	72 (34)
Current	274 (18)	41 (19)
Body mass index, kg/m^2	30.2±5.9	30.0±5.9
Diabetes mellitus	257 (17)	46 (22)
Fasting glucose, mg/dL	100±32	102±33
Hypertension	897 (59)	134 (63)
Systolic BP, mm Hg	132±22	131±21
Diastolic BP, mm Hg	74±11	75±10
Use of antihypertensive medications	757 (49)	116 (55)
Total cholesterol, mg/dL	190±36	190±37
LDL, mg/dL	117±33	115±34
HDL, mg/dL	52±15	54±16
Triglyceride, mg/dL	90 [66–123]	88 [67–122]
Use of lipid-lowering medications	237 (16)	39 (18)
Moderate-vigorous PA (MET, min/wk)	4560 [2110–8640]	4560 [2258–8505]
Family history of heart disease, No. (%)	612 (43)	70 (34)
$eGFR_{CysC}$, mL/min per 1.73 m^2	89±20	89±20
UACR, mg/g	5.4 [3.1–12.5]	6.2 [3.5–15.6]
UACR ≥30 mg/g	169 (11)	33 (16)

Values are presented as mean±SD, median [interquartile range], or number (percentage). *APOL1* high-risk defined as 2 risk alleles and low-risk defined as 0 or 1 risk allele. BP indicates blood pressure; $eGFR_{CysC}$, cystatin C–based estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MET, metabolic equivalent; PA, physical activity; UACR, urine albumin to creatinine ratio.

respectively. There was no significant difference in risk for incident CHD, MI, or stroke by *APOL1* risk status (Table 3; Table S2).

Eighty individuals (15 *APOL1* high-risk and 65 *APOL1* low-risk) developed incident HF. We found that individuals with the *APOL1* high-risk genotypes were 82% more likely to develop HF compared with individuals with the low-risk genotypes (model 2: adjusted hazard ratio, 1.82; 95% CI, 1.01–3.28). This association was not fully explained by differences in baseline hypertension status and markers of kidney function. Adding hypertension only slightly attenuated the *APOL1*-associated risk for incident HF (model 3a: adjusted hazard ratio, 1.80; 95% CI, 1.00–3.24), whereas adding cystatin C–based estimated GFR and UACR did not change the association (model 3b: adjusted hazard ratio, 1.86; 95% CI, 1.03–3.35; Table 4). Finally, in sensitivity analyses, similar trends were noted when using an additive genetic model (model 2: adjusted hazard ratio, 1.41; 95% CI, 1.01–1.97), whereas no statistically significant association was detected when using a dominant genetic model (Table S3).

Discussion

In this study of black people without baseline clinical CVD, we report that the *APOL1* risk variants were not associated with subclinical measures of CVD, including CAC, CIMT, and left ventricular mass or incident clinical atherosclerotic events. On the other hand, we found that the *APOL1* high-risk genotypes were significantly associated with an increased risk for incident HF compared with the low-risk genotypes. This association was not explained by the presence of hypertension or kidney function abnormalities at baseline. Our results add to the growing body of literature on the potential role of *APOL1* in atherosclerotic CVD and HF.

To date, few studies have examined whether the *APOL1* risk variants relate to subclinical CVD. In both JHS (Jackson Heart Study) and AA-DHS (African American-Diabetes Heart Study), individuals with *APOL1* risk variants were noted to have less calcium plaque burden in their coronary (JHS, 2 versus 0 risk alleles) and carotid (AA-DHS, 1 or 2 versus 0 risk alleles) arteries.[21,23] We similarly found that *APOL1* high-risk individuals were less likely to have CAC present compared with low-risk individuals; however, our results were not statistically significant. Varied modeling strategies and populations across the 3 studies may account for these differences. We also reported that the *APOL1* high-risk genotypes were not associated with CIMT severity. These findings are consistent with CHS (Cardiovascular Health Study), a cohort of older individuals, and the only other study we are aware of that has examined this potential association.[22] In addition, we found no significant association between the *APOL1* risk variants and left ventricular mass.

Table 2. Cross-Sectional Associations of *APOL1* Risk Variants With Subclinical Cardiovascular Disease Measures at the Baseline Examination in MESA

	No.	CAC >0	Model 1 RR (95% CI)	Model 2 RR (95% CI)
CAC				
APOL1 low-risk	1533	681 (44%)	1.00 (reference)	1.00 (reference)
APOL1 high-risk	213	77 (36%)	0.81 (0.67–0.98)	0.88 (0.75–1.03)

	No.	Geometric mean±SD	Model 1 RD* (95% CI)	Model 2 RD* (95% CI)
APOL1 low-risk	681	66±7	1.00 (reference)	1.00 (reference)
APOL1 high-risk	77	69±6	1.54 (0.98–2.41)	1.41 (0.93–2.16)

	No.	Mean±SD	Model 1 β (95% CI)	Model 2 β (95% CI)
CIMT				
APOL1 low-risk	1533	0.90±0.19 mm	0 (reference)	0 (reference)
APOL1 high-risk	213	0.92±0.20 mm	0.02 (−0.01 to 0.05)	0.01 (−0.02 to 0.04)
Left ventricular mass				
APOL1 low-risk	1054	158±41 g	0 (reference)	0 (reference)
APOL1 high-risk	152	162±44 g	4.29 (−3.26 to 11.84)	0.69 (−5.58 to 6.96)

APOL1 high-risk defined as 2 risk alleles and low-risk defined as 0 to 1 risk alleles. Model 1: unadjusted; model 2: adjusted for age, sex, and African ancestry. CI indicates confidence interval; CIMT, carotid-intimal media thickness; MESA, Multi-Ethnic Study of Atherosclerosis; RD, relative difference; RR, relative risk.
*Relative difference in geometric mean coronary artery calcification (CAC) score for *APOL1* high- vs low-risk, as estimated from a linear regression with ln(CAC score) as the dependent variable.

In further analyses, we considered clinical atherosclerotic CVD separate from congestive HF because their underlying disease mechanisms likely differ. We found no association between the *APOL1* risk variants and incident MI, coronary heart disease, or stroke. These findings are in accordance with results from the ARIC (Atherosclerosis Risk in Communities) study and SPRINT trial, which also reported no significant association between the *APOL1* high-risk variants and incident or prevalent CVD, respectively.[20,25] On the other hand, the JHS and WHI (Women's Health Initiative) both demonstrated a significantly increased risk for a major adverse cardiovascular event among people with 2 versus 0 *APOL1* high-risk alleles,[21] whereas the CHS noted an increased risk of MI but not stroke or cardiovascular

Table 3. Association of *APOL1* Risk Variants With Incident Cardiovascular Disease Events in MESA

	No.	Rate % Per Year	Model 1 HR (95% CI)	Model 2 HR (95% CI)
Incident CHD (composite)				
APOL1 low-risk	1532	0.7	1.00 (reference)	1.00 (reference)
APOL1 high-risk	213	0.7	0.97 (0.54–1.73)	0.99 (0.55–1.78)
Incident MI				
APOL1 low-risk	1532	0.2	1.00 (reference)	1.00 (reference)
APOL1 high-risk	213	0.3	1.24 (0.52–2.94)	1.23 (0.51–2.96)
Incident stroke				
APOL1 low-risk	1532	0.3	1.00 (reference)	1.00 (reference)
APOL1 high-risk	213	0.4	1.00 (0.45–2.20)	1.02 (0.46–2.25)

Coronary heart disease (CHD) defined by myocardial infarction (MI), definite or probable angina (if followed by revascularization), resuscitated cardiovascular arrest, and CHD death. *APOL1* high-risk defined as 2 risk alleles and low-risk defined as 0 or 1 risk allele. Model 1: unadjusted; model 2: adjusted for age, sex, and African ancestry. CI indicates confidence interval; HR, hazard ratio; MESA, Multi-Ethnic Study of Atherosclerosis.

Table 4. Association of *APOL1* Risk Variants With Incident HF in MESA

	APOL1 Low-Risk	*APOL1* High-Risk
Incidence Rate	0.4% Per Year	0.7% Per Year
	HR (95% CI)	HR (95% CI)
Model 1: unadjusted	1.00 (reference)	1.76 (0.98–3.15)
Model 2: adjusted for age, sex, and African ancestry	1.00 (reference)	1.82 (1.01–3.28)
Model 3a: Model 2+hypertension only	1.00 (reference)	1.80 (1.00–3.24)
Model 3b: model 2+$eGFR_{CysC}$+UACR only	1.00 (reference)	1.86 (1.03–3.35)
Model 3c: model 2+hypertension+ $eGFR_{CysC}$+UACR	1.00 (reference)	1.84 (1.02–3.32)

APOL1 high-risk defined as 2 risk alleles and low-risk defined as 0 or 1 risk allele. Estimated glomerular filtration rate was measured at examinations 1, 3, 4, and 5. Urine albumin to creatinine ratio (UACR) was measured at examinations 1, 2, 3, and 5. CI indicates confidence interval; $eGFR_{CysC}$, estimated cystatin C–based glomerular filtration rate; HF, heart failure; HR, hazard ratio; MESA, Multi-Ethnic Study of Atherosclerosis.

mortality.[22] The reasons for these discrepant findings are unclear, although differences in study populations likely contribute, including varying ages and comorbidities.[20–22,25]

Our study is one of few to examine the association of *APOL1* with incident HF. We found that people with the *APOL1* high-risk genotypes were more likely to develop HF compared with the low-risk genotypes. Prior studies have demonstrated that incident HF is much more common in blacks compared with whites.[26,27] While this difference in risk has primarily been attributed to the higher burden of traditional cardiovascular risk factors among blacks, perhaps risk variants in the *APOL1* gene contribute as well.[26,27,29] Upon further investigation, we found that this association between *APOL1* high-risk status and incident HF was only slightly attenuated by hypertension and not at all by kidney function markers. *APOL1* expression is driven by inflammation, and its protein product has been found in endothelial and vascular smooth muscle cells within the kidney.[16,55] Among individuals with decompensated HF, different variants in the *APOL1* gene have been associated with differential responsiveness to furosemide-based diuretic regimens, and whether differences in response to medications may explain observed associations is not known.[56] Perhaps the *APOL1* risk variants increase risk for HF via endothelial dysfunction or abnormalities in cardiac remodeling. Alternatively, the *APOL1* risk variants may be associated with HF caused by effects on the kidney that are not sufficiently captured by traditional clinical markers or by causing defects in sodium handling. Our significant results for congestive HF should be interpreted with caution. Although increased left ventricular mass would have potentially explained the association between the *APOL1* risk variants and incident HF risk in MESA,[57] we did not find an association between the risk variants and left ventricular mass. Unlike our study, the CHS reported no difference in risk of incident congestive HF between *APOL1* high- and low-risk individuals. The CHS cohort, however, consisted of much older individuals with a mean age of ≈73 years.[22] Further studies are needed to clarify the role of the *APOL1* high-risk variants in HF, and the findings of this study need replication. Distinctions should also be made between HF with preserved versus reduced ejection fraction, as the pathogeneses of these 2 types of HF are different.

Study Strengths and Limitations

Our study has several strengths. First, we utilized data from a well-described cohort of individuals. Second, follow-up was extensive, with a mean duration of ≈11 years. Third, CAC, CIMT, and left ventricular mass were collected in a standardized manner while all clinical atherosclerotic CVD and HF events were adjudicated using prespecified criteria. Finally, our study population was free of clinical CVD at baseline, thus allowing us to examine the variants' associations with first incident CVD event. Limitations include the relatively small number of events, limiting our power to detect small to moderate effect sizes. In addition, our analyses on subclinical CVD were cross-sectional. Still, our findings on CAC, CIMT, and left ventricular mass provide insight on potential mechanisms by which the *APOL1* risk variants are hypothesized to cause CVD. While it is possible to have copy number variation at the *APOL1* locus, these are very rare, and thus we are unable to test associations of additional possible genotypes with outcomes. Finally, the multiple models could lead to multiple comparisons problems and subsequent false-positive findings.

Conclusions

We report that the *APOL1* high-risk variants are not associated with subclinical or clinical atherosclerotic CVD; however, they may be associated with an increased risk of incident HF. Given that the *APOL1* risk variants are associated with chronic kidney disease, a known risk factor for CVD and HF, clarifying the role of *APOL1* high-risk alleles in subclinical, clinical atherosclerotic CVD, and HF is of utmost importance.

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Disclosures

Chen previously owned stock in Pfizer Pharmaceuticals. Peralta owns stock in and is a consultant for Cricket Health, Inc. and previously was a consultant for Vital Labs, Inc. The other authors have nothing to disclose.

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SUPPLEMENTAL MATERIAL

Table S1. Cross-sectional associations of *APOL1* risk variants with subclinical cardiovascular disease measures at the baseline exam in the Multi-Ethnic Study of Atherosclerosis (MESA) using additive (per additional *APOL1* risk allele) and dominant (0 versus 1-2 *APOL1* risk alleles) genetic models.

Coronary Artery Calcification (CAC)				
			Model 1	**Model 2**
	n	**CAC >0**	**RR (95% CI)**	**RR (95% CI)**
Per additional *APOL1* risk allele	1746	758 (43%)	0.94 (0.87, 1.01)	0.96 (0.90, 1.03)
0 *APOL1* risk alleles	762	339 (45%)	1.00 (ref)	1.00 (ref)
1-2 *APOL1* risk alleles	984	419 (43%)	0.96 (0.86, 1.07)	0.98 (0.89, 1.08)
			Model 1	**Model 2**
	n	**Geometric mean (SD)**	**RD* (95% CI)**	**RD* (95% CI)**
Per additional *APOL1* risk allele	758	69 ± 6	1.13 (0.91, 1.39)	1.14 (0.93, 1.39)
0 *APOL1* risk alleles	339	65 ± 6	1.00 (ref)	1.00 (ref)
1-2 *APOL1* risk alleles	419	72 ± 6	1.05 (0.80, 1.38)	1.10 (0.85, 1.43)
Carotid Intimal-Media Thickness (CIMT)				
			Model 1	**Model 2**
	n	**Mean (SD)**	**β (95% CI)**	**β (95% CI)**
Per additional *APOL1* risk allele	1746	0.91 ± 0.19 mm	0.004 (-0.01, 0.02)	-0.0001 (-0.01, 0.01)
0 *APOL1* risk alleles	762	0.91 ± 0.19 mm	0 (ref)	0 (ref)
1-2 *APOL1* risk alleles	984	0.90 ± 0.20 mm	0.001 (-0.02, 0.02)	-0.004 (-0.02, 0.01)
Left Ventricular Mass				
			Model 1	**Model 2**
	n	**Mean (SD)**	**β (95% CI)**	**β (95% CI)**
Per additional *APOL1* risk allele	1206	159 ± 42 g	1.35 (-2.30, 4.99)	0.62 (-2.44, 3.68)
0 *APOL1* risk alleles	526	158 ± 41 g	0 (ref)	0 (ref)
1-2 *APOL1* risk alleles	680	159 ± 42 g	0.65 (-4.34, 5.63)	0.85 (-3.33, 5.03)

Abbreviations: RR=relative risk; RD=relative difference; CI=confidence interval; ref=reference.

Model 1: unadjusted; Model 2: adjusted for age, sex, and African ancestry.

*Relative difference in geometric mean CAC score for *APOL1* high- vs. low-risk, as estimated from a linear regression with ln(CAC score) as the dependent variable.

Table S2. Association of *APOL1* risk variants with incident cardiovascular disease events in the Multi-Ethnic Study of Atherosclerosis (MESA) using additive (per additional *APOL1* risk allele) and dominant (0 versus 1-2 *APOL1* risk alleles) genetic models.

Incident Coronary Heart Disease (Composite)				
			Model 1	**Model 2**
	n	**# of events**	**HR (95% CI)**	**HR (95% CI)**
Per additional *APOL1* risk allele	1745	125	0.92 (0.70, 1.21)	0.96 (0.73, 1.28)
0 *APOL1* risk alleles	762	59	1.00 (ref)	1.00 (ref)
1-2 *APOL1* risk alleles	983	66	0.87 (0.60, 1.26)	0.94 (0.64, 1.37)
Incident Myocardial Infarction				
			Model 1	**Model 2**
	n	**# of events**	**HR (95% CI)**	**HR (95% CI)**
Per additional *APOL1* risk allele	1745	47	1.18 (0.76, 1.84)	1.23 (0.79, 1.92)
0 *APOL1* risk alleles	762	19	1.00 (ref)	1.00 (ref)
1-2 *APOL1* risk alleles	983	28	1.25 (0.67, 2.33)	1.35 (0.72, 2.56)
Incident Stroke				
			Model 1	**Model 2**
	n	**# of events**	**HR (95% CI)**	**HR (95% CI)**
Per additional *APOL1* risk allele	1745	65	1.06 (0.73, 1.55)	1.09 (0.74, 1.60)
0 *APOL1* risk alleles	762	27	1.00 (ref)	1.00 (ref)
1-2 *APOL1* risk alleles	983	38	1.12 (0.66, 1.88)	1.16 (0.68, 1.98)

Coronary Heart Disease (CHD) defined by myocardial infarction, definite or probable angina (if followed by revascularization), resuscitated cardiovascular arrest, and CHD death.

Model 1: unadjusted; Model 2: adjusted for age, sex, and African ancestry.

Abbreviations: CI=confidence interval; ref=reference.

Table S3. Association of *APOL1* risk variants with incident heart failure in the Multi-Ethnic Study of Atherosclerosis (MESA) using additive (per additional *APOL1* risk allele) and dominant (0 versus 1-2 *APOL1* risk alleles) genetic models.

	Additive Genetic Model	**Dominant Genetic Model**
	Per additional *APOL1* risk allele	1-2 vs. 0 *APOL1* risk alleles
	Hazard Ratio (95% CI)	**Hazard Ratio (95% CI)**
Model 1: unadjusted	1.36 (0.98, 1.88)	1.35 (0.84, 2.16)
Model 2: adjusted for age, sex, and African ancestry	1.41 (1.01, 1.97)	1.43 (0.88, 2.31)
Model 3a: Model 2 + hypertension only	1.41 (1.01, 1.96)	1.43 (0.88, 2.32)
Model 3b: Model 2 + $eGFR_{CysC}$ + UACR only	1.45 (1.04, 2.03)	1.49 (0.92, 2.44)
Model 3c: Model 2 + hypertension + $eGFR_{CysC}$ + UACR	1.45 (1.04, 2.02)	1.49 (0.91, 2.43)

Abbreviations: CI=confidence interval; ref=reference; $eGFR_{CysC}$=estimated cystatin-c based glomerular filtration rate; UACR=urine albumin-to-creatinine ratio.

eGFR was measured at Exams 1, 3, 4, and 5. UACR was measured at Exams 1, 2, 3, and 5.