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APOL1 Risk Variants and Subclinical Cardiovascular Disease in Incident Hemodialysis Patients

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Introduction: To better understand the impact of *APOL1* risk variants in end-stage renal disease (ESRD) we evaluated associations of *APOL1* risk variants with subclinical cardiovascular disease (CVD) and mortality among African Americans initiating hemodialysis and enrolled in the Predictors of Arrhythmic and Cardiovascular Risk in ESRD cohort study.

Methods: We modeled associations of *APOL1* risk status (high = 2; low = 0/1 risk alleles) with baseline subclinical CVD (left ventricular [LV] hypertrophy; LV mass; ejection fraction; coronary artery calcification [CAC]; pulse wave velocity [PWV]) using logistic and linear regression and all-cause or cardiovascular mortality using Cox models, adjusting for age, sex, and ancestry. In sensitivity analyses, we further adjusted for systolic blood pressure and Charlson Comorbidity Index.

Results: Of 267 African American participants successfully genotyped for *APOL1*, 27% were high-risk carriers, 41% were women, and mean age was 53 years. At baseline, *APOL1* high- versus low-risk status was independently associated with 50% and 53% lower odds of LV hypertrophy and CAC, respectively, and 10.7% lower LV mass. These associations were robust to further adjustment for comorbidities but not systolic blood pressure. *APOL1* risk status was not associated with all-cause or cardiovascular mortality (mean follow-up 2.5 years).

Conclusion: Among African American patients with incident hemodialysis, *APOL1* high-risk status was associated with better subclinical measures of CVD but not mortality.

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CVD is a common complication of chronic kidney disease (CKD) associated with increased morbidity and mortality. In a sample of Medicare patients, CVD prevalence was approximately 2-fold higher in those with CKD compared with those without CKD (65% vs. 32%, respectively).[1] Among patients with ESRD on hemodialysis, prevalence of CVD is even higher at 71%.[1] Cardiovascular disease and CKD are closely intertwined: the presence of both portends worse short and long-term survival.[1]

Studies to date have consistently demonstrated that risk variants in the *APOL1* gene confer an increased risk for kidney disease progression.[2–6] The *APOL1* risk haplotypes, known as G1 and G2, are found almost exclusively in persons of African ancestry and are associated with focal segmental glomerulosclerosis, HIV-associated nephropathy, and hypertension-attributed CKD.[2–6] Early studies suggested that the *APOL1* risk variants also may be associated with adverse cardiovascular outcomes[7,8]; however, a recent meta-analysis of 8 pre-ESRD cohorts reported no

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association between *APOL1* high-risk status (2 risk alleles) and incident clinical CVD.[9] In another large cohort of African American US veterans (mean estimated glomerular filtration rate ~86–90 ml/min per 1.73 m^2), *APOL1* high-risk status was modestly associated with incident coronary artery disease, although this was thought to be mediated via the variants' associations with CKD.[10] A phenome-wide association analysis of African American individuals (median estimated glomerular filtration rate ~67–79 ml/min per 1.73 m^2 in the Penn Medicine Biobank; 6% with ESRD on dialysis in the Vanderbilt BioVU) also reported that the *APOL1* risk variants were not independently associated with CVD phenotypes.[11] These studies primarily consisted of individuals with normal kidney function or non–dialysis-dependent CKD. The significance of *APOL1* risk variants after the development of ESRD is less clear. A single study implicated that prevalent hemodialysis patients with *APOL1* high-risk status had a survival benefit compared with those with low-risk status (0/1 risk allele).[12]

The transition from CKD to ESRD is a particularly vulnerable period for CVD complications. With dialysis initiation comes sudden, marked changes in serum electrolyte levels, volume status, and blood pressure, all of which can further increase CVD risk.[13] We aimed to study the associations of *APOL1* risk variants with subclinical CVD and mortality in a cohort of black incident hemodialysis patients. We hypothesized that participants with *APOL1* high-risk status would have more subclinical CVD at baseline and, thus, increased risks of all-cause and cardiovascular mortality compared with their counterparts with *APOL1* low-risk status.

METHODS

Study Population

The Predictors of Arrhythmic and Cardiovascular Risk in End-Stage Renal Disease (PACE) study was a prospective observational cohort designed to investigate arrhythmic and sudden cardiac death risks among incident hemodialysis patients. Details regarding the study have previously been reported.[14] Briefly, participants, aged ≥18 years, were recruited between November 2008 and August 2012 from 27 outpatient (25 free-standing; 2 hospital-based) hemodialysis units in Baltimore, Maryland, and the surrounding area.[14,15] Incident hemodialysis was defined as initiation of regular outpatient thrice-weekly hemodialysis within 6 months of enrollment. Exclusion criteria included home hemodialysis or peritoneal dialysis; presence of a pacemaker and/or automatic implantable cardioverter-defibrillator; cancer (except nonmelanoma skin cancer); pregnant or nursing mothers; individuals in hospice, a skilled nursing facility, or prison; and health conditions that might interfere with study participation (e.g., dementia or psychosis). Informed consent was obtained from all participants. The study was approved by institutional review boards of the Johns Hopkins University School of Medicine and MedStar Health.[14] Among 393 self-reported African American participants, 371 consented to genetic testing, and 267 were successfully genotyped for ancestry informative markers and *APOL1* risk variants (Supplementary Figure S1).

Genotyping

The primary predictor was *APOL1* risk status, defined by a recessive genetic model. At baseline and annually thereafter, biospecimens were collected and stored at −80°C. DNA was extracted from whole blood (n = 222) and buffy coat (n = 45) samples by LGC Genomics (Beverly, MA). Genotyping was performed at the Frederick National Laboratory for Cancer Research (Frederick, MD) using custom Taqman assays for *APOL1* (rs60910134, rs73885319, and rs71785313) and using the Infinium QC Array-24 v1.0 (Illumina; San Diego, CA) for 15,949 ancestry informative markers, of which 15,030 passed quality control. Ancestry was estimated by principal components (PCs) analysis (performed by The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, Canada) using the SmartPCA package (version 10210) of Eigenstrat 5.0.1[16,17] with the 1000 Genomes Project as the reference dataset. Based on the scree plot of eigenvalues versus PC index, the first 2 PCs had the strongest effects. For *APOL1*, high-risk status was defined as having 2 risk alleles; low-risk status was defined as having 0 or 1 risk allele.

Outcomes

All baseline cardiac assessments were obtained by trained personnel on nondialysis days at the Johns Hopkins Institute for Clinical and Translational Research clinic. LV hypertrophy, mass, and ejection fraction were determined from echocardiograms (Toshiba Artida; Toshiba, Tokyo, Japan) read centrally by the Johns Hopkins Cardiovascular Laboratory. Using M-mode of the parasternal short axis view, LV mass was estimated by 0.8*(1.04*([LVIDD + PWTD + IVSTD]^3 − [LVIDD]^3)) + 0.6 g, where LVIDD = LV internal diameter, PWTD = posterior wall thickness, and IVSTD = interventricular septum thickness in diastole. LV hypertrophy was defined as a LV mass index ≥116 g/m^2 for men and ≥104 g/m^2 for women. Ejection fraction was calculated by (end diastolic − end systolic volume)/(end diastolic volume) × 100%. Quantified by method of Agatston, CAC was measured

Table 1. Baseline characteristics of African American PACE participants included in study, by *APOL1* genotype status

	All (n = 267)	*APOL1* high-risk (n = 73)	*APOL1* low-risk (n = 194)	P value
Age, y	52.6 ± 12.0	49.0 ± 13.2	53.9 ± 11.3	<0.01
Female, *n* (%)	109 (41)	29 (40)	80 (41)	0.82
Current smoker, *n* (%)	77 (29)	21 (29)	56 (29)	0.97
History of hypertension, *n* (%)	267 (100)	73 (100)	194 (100)	—
History of diabetes, *n* (%)	150 (56)	26 (36)	124 (64)	<0.01
History of atrial fibrillation, *n* (%)	70 (26)	19 (26)	51 (26)	0.97
Prevalent coronary artery disease, *n* (%)	85 (32)	19 (26)	66 (34)	0.21
Prevalent cerebrovascular disease, *n* (%)	65 (24)	19 (26)	46 (24)	0.69
Prevalent congestive heart failure, *n* (%)	100 (37)	23 (32)	77 (40)	0.22
Body mass index, kg/m^2	29.2 ± 8.3	29.6 ± 8.1	29.0 ± 8.4	0.61
Systolic blood pressure, mm Hg	138 ± 26	132 ± 24	140 ± 26	0.03
Diastolic blood pressure, mm Hg	77 ± 14	77 ± 15	77 ± 14	0.94
Pulse pressure, mmHg	61 ± 18	56 ± 16	63 ± 19	<0.01
Days from dialysis initiation to baseline cardiac assessment	121 ± 61	128 ± 61	119 ± 62	0.31
Cause of ESRD, *n* (%)				
Glomerulonephritis	37 (14)	18 (25)	19 (10)	<0.01
Hypertension	69 (26)	22 (30)	47 (24)	
Diabetes	92 (34)	15 (21)	77 (40)	
Other	44 (16)	15 (21)	29 (15)	
Unknown	25 (9)	3 (4)	22 (11)	
Kt/V average over 3 mo	1.78 ± 0.35	1.78 ± 0.34	1.77 ± 0.35	0.89
Intradialytic weight change average over 3 mo, kg	2.3 ± 0.9	2.2 ± 0.8	2.3 ± 0.9	0.34
Total cholesterol, mg/dl	171 ± 44	167 ± 35	172 ± 47	0.40
High-density lipoprotein, mg/dl	54 ± 18	54 ± 17	55 ± 19	0.67
Triglycerides, mg/dl	129 ± 61	134 ± 68	127 ± 59	0.43
Low-density lipoprotein, mg/dl	90 ± 39	87 ± 31	92 ± 41	0.29
Use of anti-hypertensive medications, *n* (%)	215 (97)	55 (95)	160 (98)	0.31
Use of statins, *n* (%)	98 (44)	26 (45)	72 (44)	0.90
Use of aspirin, *n* (%)	90 (41)	18 (31)	72 (44)	0.09
Vascular access, n (%)				0.02
Arteriovenous fistula	67 (25)	15 (21)	52 (27)	
Arteriovenous graft	11 (4)	7 (10)	4 (2)	
Venous catheter	187 (71)	51 (70)	136 (71)	
Charlson Comorbidity Index, points	5 ± 2	5 ± 2	5 ± 2	0.37
LV hypertrophy, *n* (%)	176 (72)	40 (63)	136 (76)	0.04
LV mass, g	272 [212 to 345]	252 [198 to 327]	278 [219 to 355]	0.10
Ejection fraction, %	66.0 ± 11.7	64.6 ± 9.1	66.5 ± 12.4	0.21
CAC >0, *n* (%)	109 (54)	18 (34)	91 (61)	<0.01
CAC, Agatston score[a]	173 [21 to 607]	199 [47 to 380]	171 [19 to 617]	0.87
Pulse wave velocity, m/s	9.8 [7.9 to 12.5]	8.7 [7.4 to 11.3]	10.5 [8.3 to 13.1]	<0.01

CAC, coronary artery calcification; ESRD, end-stage renal disease; HIV, human immunodeficiency virus; LV, left ventricular; PACE, Predictors of Arrhythmic and Cardiovascular Risk in End-Stage Renal Disease.

[a]Among participants with CAC>0. Values presented as number (%), mean ± SD, or median [interquartile range]. *APOL1* genotype status defined by a recessive genetic model: high-risk = 2 risk alleles and low-risk = 0–1 risk alleles.

Missing values for the following variables: smoking (n = 1), body mass index (n = 2), systolic blood pressure (n = 18), diastolic blood pressure (n = 18), pulse pressure (n = 18), days from dialysis initiation to baseline cardiac assessment (n = 19), Kt/V (n = 23), intradialytic weight change (n = 23), total cholesterol (n = 19), high-density lipoprotein (n = 19), triglycerides (n = 19), low-density lipoprotein (n = 20), anti-hypertensive medication use (n = 45), statin use (n = 45), aspirin use (n = 45), vascular access (n = 2), LV hypertrophy (n = 24), LV mass (n = 23), ejection fraction (n = 23), CAC (n = 66), pulse wave velocity (n = 75).

using multidetector computed tomography and angiography (Toshiba Aquilon 32; Toshiba)[18,19] and CAC presence defined by an Agatston score >0. PWV of the right carotid and right femoral arteries was assessed using the Sphygmocor PVx system (Atcor Medical, West Ryde, New South Wales, Australia) after 5 minutes of rest with the patient in supine position. Measurements were obtained by trained personnel following a standardized protocol that included real-time quality control checks.[14]

In addition to annual clinic visits (for up to 4 years), study coordinators contacted dialysis units and participants semi-annually to collect information on hospitalizations and vital status. On notification of a death, records from recent hospitalization or emergency room visits and the Centers for Medicaid and Medicare Services death notification form (CMS-2746) were obtained. Next of kin were also interviewed. All deaths were adjudicated by the PACE Endpoint Committee. Cardiovascular mortality was defined as death

Table 2. Associations of *APOL1* genotype status with subclinical cardiovascular disease at baseline in PACE, comparing *APOL1* high- versus low-risk status

	Unadjusted	Adjusted for age, sex and ancestry	Adjusted for age, sex, ancestry, and CCI	Adjusted for age, sex, ancestry, and SBP
Odds ratio (95% confidence interval) ***P* value**				
LV hypertrophy (n = 243)	0.53 (0.29 to 0.97) 0.04	0.50 (0.26 to 0.94) 0.03	0.51 (0.27 to 0.96) 0.04	0.54[b] (0.28 to 1.03) 0.06
CAC >0 (n = 201)	0.32 (0.17 to 0.62) 0.001	0.47 (0.22 to 0.98) 0.04	0.47 (0.22 to 0.98) 0.04	0.51[b] (0.24 to 1.07) 0.08
β (95% confidence interval) ***P* value**				
Ejection fraction (n = 244)	−1.84 (−5.18 to 1.50) 0.28	−1.47 (−4.93 to 1.99) 0.41	−1.69 (−5.13 to 1.75) 0.34	−1.67[b] (−5.20 to 1.86) 0.35
% Difference (95% confidence interval) ***P* value**				
CAC[a] (n = 109)	7.37 (−62.40 to 206.62) 0.89	68.40 (−36.52 to 346.76) 0.29	81.88 (−30.93 to 378.95) 0.22	61.59 (−38.80 to 326.61) 0.33
LV mass (n = 244)	−7.94 (−16.58 to 1.60) 0.10	−10.69 (−18.99 to −1.54) 0.02	−10.56 (−18.90 to −1.36) 0.03	−9.00[b] (−17.40 to 0.26) 0.06
Pulse wave velocity (n = 192)	−12.98 (−20.85 to −4.32) 0.004	−7.86 (−16.08 to 1.16) 0.09	−7.72 (−15.92 to 1.29) 0.09	−4.99 (−12.91 to 3.64) 0.25

CAC, coronary artery calcification; CCI, Charlson Comorbidity Index; LV, left ventricular; PACE, Predictors of Arrhythmic and Cardiovascular Risk in End-Stage Renal Disease; SBP, systolic blood pressure.
[a]Among individuals with CAC >0.
[b]Number of participants is n-1.
APOL1 risk status defined by a recessive genetic model: high-risk = 2 risk alleles and low-risk = 0–1 risk alleles.

attributed to sudden cardiac death, arrhythmia, or ischemic cardiovascular or cerebrovascular event.[14]

Covariates

Sociodemographic and clinical data were collected at baseline from questionnaires and medical records. Race was self-reported. Cause of ESRD was determined from review of medical records, kidney biopsy reports, and the CMS-2728 form. Baseline comorbidity was evaluated using the Charlson Comorbidity Index and adjudicated by the PACE Endpoint Committee. All participants underwent a baseline physical examination on a nondialysis day, including measurements of height and weight. Three seated resting blood pressures, measured in a standardized manner using an oscillometric machine, were averaged. Lipids were measured from fasting (≥8 hours) biospecimens using the Roche Integra Analyzer (Indianapolis, IN) at the Laboratory for Clinical Biochemistry Research in Vermont.[14]

Statistical Analyses

Baseline characteristics were compared by *APOL1* risk status using Student's *t*-test or Wilcoxon rank-sum test for continuous variables and χ^2 test for categorical variables. Continuous outcomes that were skewed (LV mass, CAC, PWV) were natural-log transformed to achieve a more normal distribution. In cross-sectional analyses, the associations of *APOL1* risk status with LVH and CAC (as binary variables) were assessed using logistic regression, and with LV mass, CAC, ejection fraction, and PWV (as continuous variables) using linear regression. In time to event analyses, the associations of *APOL1* risk status with all-cause and cardiovascular mortality were assessed using Cox proportional hazards models. Administrative censoring occurred on June 30, 2014, and the proportional hazards assumption was checked using Schoenfeld residuals. The following models were constructed: (1) Unadjusted; (2) Model 1 adjusted for age, sex, and ancestry (PCs 1 and 2); (3) Model 2 adjusted for age, sex, ancestry, and Charlson Comorbidity Index; and (4) Model 3 adjusted for age, sex, ancestry, and systolic blood pressure. Given that our primary exposure was a gene, we treated model 1 as our final model. To assess the robustness of our findings, we further adjusted for Charlson Comorbidity Index as a marker of general health in model 2 and systolic blood pressure as a potential mediator in model 3.[20] For the outcomes of ejection fraction, LVH, and LV mass, we further adjusted for average intradialytic weight change (in addition to age, sex, and ancestry), as a surrogate marker of volume removed with hemodialysis, in the 3 months preceding baseline cardiovascular measurement. In sensitivity analyses, we assessed for effect modification by history of diabetes (yes vs. no) for subclinical CVD and by age (<55 vs. ≥55 years), history of diabetes, and cause of ESRD (diabetes vs. other) for all-cause and cardiovascular mortality using interaction terms of each with *APOL1* risk status. Because

Table 3. Associations of *APOL1* high- versus low-risk genotypes with subclinical cardiovascular disease at baseline in PACE, by history of diabetes

	Diabetes[a]		No diabetes[a]		
	n	Odds Ratio (95% CI) *P* value	*n*	Odds Ratio (95% CI) *P* value	*P*-interaction[b]
LV hypertrophy	134	0.52 (0.19 to 1.44) 0.21	109	0.43 (0.17 to 1.06) 0.07	0.99
CAC >0	110	0.41 (0.21 to 0.80) 0.01	91	1.00 (0.50 to 1.99) 1.00	0.07
		β (95% CI) *P* value		β (95% CI) *P* value	
Ejection fraction	135	0.15 (−5.88 to 6.18) 0.96	109	−2.26 (−6.69 to 2.18) 0.32	0.41
		% Difference (95% CI) *P* value		% Difference (95% CI) *P* value	
CAC[c]	61	213.50 (−65.43 to 2743.09) 0.30	48	108.58 (−34.45 to 563.66) 0.21	0.65
LV mass	135	−3.57 (−17.47 to 12.67) 0.65	109	−15.07 (−26.07 to −2.44) 0.02	0.35
Pulse wave velocity	98	−5.64 (−19.10 to 10.06) 0.46	94	−3.80 (−13.06 to 6.45) 0.45	0.77

CAC, coronary artery calcification; CCI, Charlson Comorbidity Index; CI, confidence interval; LV, left ventricular; PACE, Predictors of Arrhythmic and Cardiovascular Risk in End-Stage Renal Disease; SBP, systolic blood pressure.
[a]Models adjusted for age, sex, and ancestry.
[b]Models adjusted for age, sex, ancestry, history of diabetes, and interaction term between *APOL1* risk status and history of diabetes.
[c]Among individuals with CAC >0.
APOL1 genotype status defined by a recessive genetic model: high-risk = 2 risk alleles and low-risk = 0–1 risk alleles.

prior studies suggested additive effects of *APOL1*,[20–22] we also considered an additive genetic model (0, 1, or 2 risk alleles). Analyses were performed using Stata 15.1 software (StataCorp LLC; College Station, TX) with $P < 0.05$ considered statistically significant.

RESULTS

Baseline Characteristics

Among 267 African American PACE participants, 27% had 2 risk alleles and 73% had 0 or 1 risk allele (Supplementary Table S1). At baseline, the *APOL1* high-risk group was younger, less likely to have diabetes, and had lower mean systolic blood pressure and pulse pressure compared with the low-risk group. Cause of ESRD also differed, with most cases attributed to hypertension and diabetes in the *APOL1* high- and low-risk groups, respectively (Table 1). Participants included in the study were younger (mean age 53 vs. 58 years; $P = .0002$), less likely to be women (41% vs. 53%; $P = 0.02$), more likely to have a history of hypertension (100% vs. 98%; $P = 0.04$), had a lower mean Kt/V (1.78 vs. 1.86; $P = 0.03$) and Charlson Comorbidity Index score (5.20 vs. 5.70; $P = 0.03$), worse lipid parameters (mean total cholesterol 171 vs. 155 mg/dl; $P = 0.02$ and mean low-density lipoprotein 90 vs. 74 mg/dl; $P = 0.03$), and were less likely to have CAC (54% vs. 74%; $P = 0.04$) with a lower median CAC (173 vs. 327 Agatston score [among those with CAC >0]; $P = 0.04$) compared with African American participants who were excluded from the study.

LV Hypertrophy and LV Mass

Among participants with available baseline echocardiogram data, 72% had LVH. Although fewer participants in the *APOL1* high-risk group had LVH compared with the low-risk group (63% vs. 76%; $P = 0.04$), median LV mass did not differ significantly between groups (252 g vs. 278 g for *APOL1* high- vs. low-risk; $P = 0.10$). Participants with the *APOL1* high-risk genotypes had 50% lower odds of LVH (95% confidence interval [CI] 0.26–0.94) and 10.7% lower LV

Table 4. Hazard risk of all-cause mortality and cardiovascular mortality in PACE, comparing *APOL1* high- versus low-risk status

	n	Events	Hazard ratio (*APOL1* high vs. low risk)	95% CI	*P* value
All-cause mortality					
Unadjusted	267	53	0.78	0.42–1.45	0.43
Adjusted for age, sex, and ancestry	267	53	0.81	0.43–1.53	0.52
Additionally adjusted for CCI	267	53	0.86	0.45–1.63	0.64
Additionally adjusted for SBP	249	49	0.75	0.38–1.50	0.42
Cardiovascular mortality					
Unadjusted	267	22	0.71	0.26–1.94	0.51
Adjusted for age, sex, and ancestry	267	22	0.65	0.23–1.79	0.40
Additionally adjusted for CCI	267	22	0.65	0.23–1.84	0.42
Additionally adjusted for SBP	249	21	0.53	0.17–1.62	0.26

CCI, Charlson Comorbidity Index; CI, confidence interval; PACE, Predictors of Arrhythmic and Cardiovascular Risk in End-Stage Renal Disease; SBP, systolic blood pressure.
Follow-up time was 2.7 years for *APOL1* high-risk group and 2.4 years and for *APOL1* low-risk group ($P = 0.12$).
APOL1 risk status defined by a recessive genetic model: high-risk = 2 risk alleles and low-risk = 0–1 risk alleles.

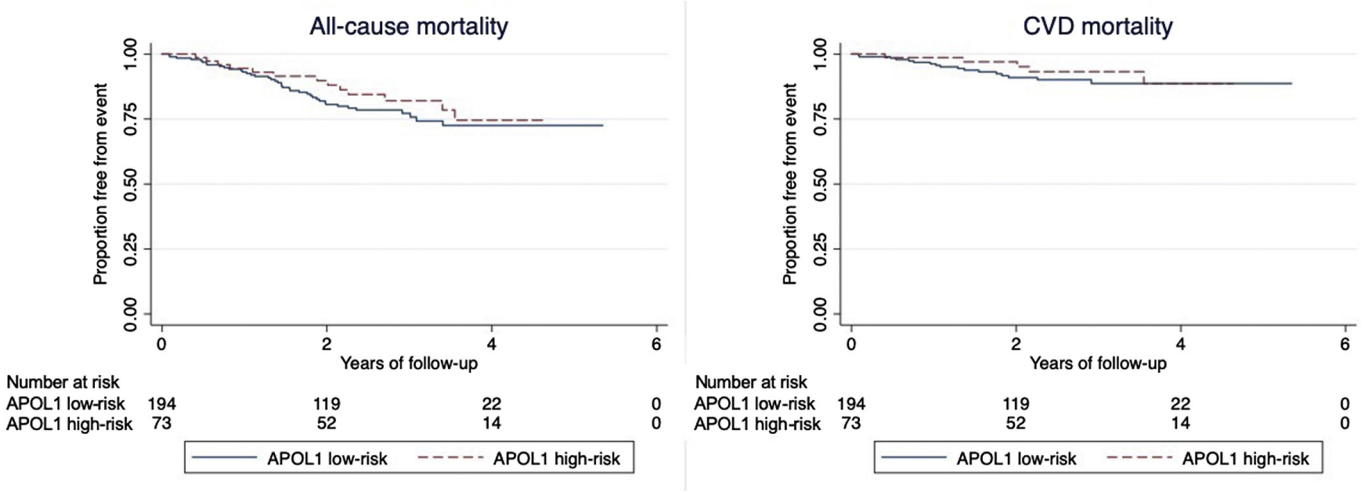

Figure 1. Kaplan-Meier survival curves for all-cause mortality and cardiovascular mortality, by *APOL1* genotype status.

mass (95% CI −18.99 to −1.54) compared with their counterparts with the low-risk genotypes, after adjusting for age, sex, and ancestry. These associations persisted after further adjustment for the Charlson Comorbidity Index but not systolic blood pressure (Table 2) and were not modified by history of diabetes (Table 3). Accounting for average intradialytic weight change did not alter the association of *APOL1* high-risk status with LVH (odds ratio 0.51; 95% CI 0.27–0.98) but did attenuate its association with LV mass (% difference −8.34; 95% CI: −16.66 to 0.80).

Coronary Artery Calcification

CAC was present in 54% of participants who underwent baseline computed tomography and angiography. A lower percentage of participants in the *APOL1* high-risk group had CAC presence compared with the low-risk group (34% vs. 61%; $P < 0.01$). Among participants with CAC, however, median Agatston scores did not differ significantly between the 2 groups (199 vs. 171 for *APOL1* high- vs. low-risk; $P = 0.87$). Participants with the *APOL1* high-risk genotypes had 53% lower odds of having CAC (95% CI 0.22–0.98) compared with those with the low-risk genotypes, after adjusting for age, sex, and ancestry (Table 2). On further investigation, this association appeared to be primarily in participants with a history of diabetes (odds ratio 0.41; 95% CI 0.21–0.80); however, formal testing for effect modification by history of diabetes did not reach statistical significance (Table 3; *P*-interaction = 0.07). The association of *APOL1* high-risk status with lower CAC remained statistically significant after further adjustment for the Charlson Comorbidity Index but not systolic blood pressure. There was no significant difference in CAC severity between the 2 *APOL1* risk groups (Table 2).

Ejection Fraction

Mean ejection fraction was 66.0% (64.6% and 66.5% in *APOL1* high- vs. low-risk groups; $P = 0.21$). The *APOL1* risk genotypes were not associated with baseline ejection fraction in any of the models (Table 2) or on further adjustment for average intradialytic weight change (β −1.56; 95% CI −5.16 to 2.03). There was no effect modification by history of diabetes (Table 3).

Pulse Wave Velocity

The median PWV was lower in the *APOL1* high- compared with low-risk group (8.7 vs. 10.5 m/s; $P < 0.01$); however, *APOL1* high-risk genotypes were not associated with PWV after adjusting for age, sex, or ancestry (Table 2), nor was there effect modification by diabetes history (Table 3).

All-cause and Cardiovascular Mortality

Over a mean follow-up of 2.5 years, risk of all-cause mortality did not differ significantly between the *APOL1* high- vs. low-risk groups (hazard ratio = 0.81; 95% CI 0.43–1.53). When specifically considering cardiovascular mortality, risk also did not differ among participants with the *APOL1* high- versus low-risk genotypes (hazard ratio = 0.65; 95% CI 0.23–1.79; Table 4; Figure 1). There was no evidence of effect modification by age, history of diabetes, or diabetes as cause of ESRD (*P*-interaction >0.05 for each).

Additive Genetic Model

When considering an additive genetic model, the odds of having LVH at baseline was 36% lower per one additional *APOL1* risk allele (95% CI 0.43–0.97), adjusting for age, sex, and ancestry. This protective association was attenuated and no longer statistically significant after further adjustment for systolic blood

pressure (odds ratio 0.67; 95% CI 0.44–1.02). When considering LV mass, each additional *APOL1* risk allele was associated with a 7.39% lower LV mass (95% CI −12.68 to −1.78) that persisted after further adjustment for the Charlson Comorbidity Index (−7.44%; 95% CI −12.73 to −1.82) or systolic blood pressure (−6.13%; 95% CI −11.47 to −0.47). There was no association between number of *APOL1* risk alleles and baseline CAC, ejection fraction, or PWV (Supplementary Table S2) or time to all-cause or cardiovascular mortality (Supplementary Table S3).

DISCUSSION

In this study of African American incident hemodialysis patients, *APOL1* high-risk status was common and paradoxically associated with better baseline measures of subclinical CVD, namely lower likelihood of LVH and CAC and lower LV mass, compared with those with low-risk status. Despite these findings, *APOL1* high-risk status was not associated with all-cause or cardiovascular mortality.

As anticipated, the prevalence of *APOL1* risk alleles is higher among persons with ESRD compared with those with predialysis CKD or the general population. Specifically, 27% of our study population had 2 *APOL1* risk alleles compared with 19% to 23% in CKD cohorts and 13% to 14% in general population cohorts.[4,7,23] Other studies of incident and prevalent chronic hemodialysis patients have reported prevalence of 37% and 29%, respectively.[12,24] In addition, we found that individuals with *APOL1* high-risk status were on average 5 years younger at the time of dialysis initiation compared with individuals with low-risk status, consistent with prior studies.[12,24,25] Taken together, these findings provide support for the aggressive nature of *APOL1*-associated kidney disease, leading to ESRD.

Subclinical CVD is common at the time of dialysis initiation,[13] with most of our participants having LVH and CAC. Surprisingly, *APOL1* high-risk status was associated with better subclinical CVD measures, specifically lower odds of CAC and LVH and lower mean LV mass. To our knowledge, this is the first study to report an association of *APOL1* risk variants with subclinical measures of CVD in ESRD. We previously reported in the Multi-Ethnic Study of Atherosclerosis, a cohort of individuals without baseline clinical CVD, that *APOL1* high-risk status was not associated with CAC or LV mass.[26] Similarly, *APOL1* high-risk status was not associated with CAC or LVH in the Coronary Artery Disease Risk in Young Adults study, another cohort of healthy young adults.[27] In the Jackson Heart Study, a population-based cohort, *APOL1* high-risk status was associated with lower Agatston scores in the left main coronary artery but not LVH.[7] In the African American–Diabetes Heart Study, presence of one *APOL1* risk allele was associated with lower calcified plaques in the carotid artery but not coronary artery or aorta.[28] Given that APOL1 protein and RNA are expressed in vascular smooth muscle and endothelial cells within the kidney,[29,30] the risk variants could conceivably protect against subclinical CVD via direct and local effects on the vasculature. Alternatively, individuals with *APOL1* high-risk status may simply represent a healthier population at hemodialysis initiation compared with their counterparts with low-risk status. In support of this, we observed that the *APOL1* high-risk group was younger and much less likely to have diabetes compared with the low-risk group. Moreover, mean blood pressure was lower in the *APOL1* high-risk group. Once we adjusted for systolic blood pressure, the protective associations of *APOL1* with CAC, LVH, and LV mass dissipated.

In our study, *APOL1* high-risk status was not associated with all-cause or cardiovascular mortality. This is in contrast to a previous study reporting an association of *APOL1* risk variants with longer dialysis survival, but only among patients with nondiabetic ESRD.[12] Their study population consisted of prevalent (~3–4 years) hemodialysis patients with *APOL1* high-risk individuals having fewer comorbidities, perhaps leading to a survival bias.[12] Consistent with our findings, *APOL1* high-risk status was not associated with all-cause mortality in a meta-analysis of 8 cohorts consisting of nonhemodialysis patients.[9]

Our study has several strengths. First, each participant underwent extensive and standardized cardiac phenotyping at baseline, enabling us to study associations of *APOL1* risk status with various measures of subclinical CVD. Second, our study population consisted of patients with ESRD who initiated hemodialysis within 6 months of enrollment. We likely had less survival bias compared with prior studies of prevalent hemodialysis patients. Third, extensive efforts were made to ensure that all deaths were captured and formally adjudicated by an endpoint committee. Our study also has limitations warranting consideration. With only 53 mortality events and relatively short follow-up of 2.5 years, we may have had limited power to detect a difference in mortality risk by *APOL1* risk status. Our analyses of subclinical CVD were cross-sectional. Given that PACE participants were recruited from the greater Baltimore area, our findings may not be generalizable to other patient populations. There were also several notable differences between participants included versus excluded from our study, which may have resulted in a selection bias. Finally, log-transforming

CAC decreased but did not completely normalize its skewed distribution; therefore, the results should be cautiously interpreted.

In conclusion, among African American incident hemodialysis patients, *APOL1* high-risk status was associated with better measures of subclinical CVD but not mortality. Additional studies are needed to confirm our findings in other ESRD populations and to better understand the clinical significance of these associations with subclinical CVD.

DISCLOSURE

MME has received an advisory panel honorarium from Astra Zeneca. All the other authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

Conception or design (TKC, RSP, MME); Genotyping (CAW, EAB-R); Analysis and interpretation of data, or both (all authors); Drafting of manuscript (TKC) or critical revision of manuscript (all authors); Providing intellectual content of critical importance to the work described (all authors); Supervision (MME).

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. *APOL1* genotype and risk allele frequencies in PACE compared with incident hemodialysis, general population, and CKD cohorts.

Table S2. Associations of *APOL1* genotype status with subclinical cardiovascular disease at baseline in PACE, per additional *APOL1* risk allele.

Table S3. Hazard risk of all-cause mortality and cardiovascular mortality in PACE, per additional *APOL1* risk allele.

Figure S1. Flowchart of study population.

STROBE Statement Checklist.

REFERENCES

1. US Renal Data System. 2018 USRDS annual data report: Epidemiology of kidney disease in the United States. Bethesda, MD: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases; 2018.
2. Genovese G, Friedman DJ, Ross MD, et al. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. *Science*. 2010;329:841–845.
3. Tzur S, Rosset S, Shemer R, et al. Missense mutations in the APOL1 gene are highly associated with end stage kidney disease risk previously attributed to the MYH9 gene. *Hum Genet*. 2010;128:345–350.
4. Parsa A, Kao WH, Xie D, et al. APOL1 risk variants, race, and progression of chronic kidney disease. *N Engl J Med*. 2013;369:2183–2196.
5. Kopp JB, Nelson GW, Sampath K, et al. APOL1 genetic variants in focal segmental glomerulosclerosis and HIV-associated nephropathy. *J Am Soc Nephrol*. 2011;22:2129–2137.
6. Larsen CP, Beggs ML, Saeed M, Walker PD. Apolipoprotein L1 risk variants associate with systemic lupus erythematosus-associated collapsing glomerulopathy. *J Am Soc Nephrol*. 2013;24:722–725.
7. Ito K, Bick AG, Flannick J, et al. Increased burden of cardiovascular disease in carriers of APOL1 genetic variants. *Circ Res*. 2014;114:845–850.
8. Mukamal KJ, Tremaglio J, Friedman DJ, et al. APOL1 genotype, kidney and cardiovascular disease, and death in older adults. *Arterioscler Thromb Vasc Biol*. 2016;36:398–403.
9. Grams ME, Surapaneni A, Ballew SH, et al. APOL1 kidney risk variants and cardiovascular disease: an individual participant data meta-analysis. *J Am Soc Nephrol*. 2019;30:2027–2036.
10. Bick AG, Akwo E, Robinson-Cohen C, et al. Association of APOL1 risk alleles with cardiovascular disease in blacks in the million veteran program. *Circulation*. 2019;140:1031–1040.
11. Bajaj A, Ihegword A, Qiu C, et al. Phenome-wide association analysis suggests the APOL1 linked disease spectrum primarily drives kidney-specific pathways. *Kidney Int*. 2020;97:1032–1041.
12. Ma L, Langefeld CD, Comeau ME, et al. APOL1 renal-risk genotypes associate with longer hemodialysis survival in

prevalent nondiabetic African American patients with end-stage renal disease. *Kidney Int*. 2016;90:389–395.

13. Bansal N. Evolution of cardiovascular disease during the transition to end-stage renal disease. *Semin Nephrol*. 2017;37:120–131.
14. Parekh RS, Meoni LA, Jaar BG, et al. Rationale and design for the Predictors of Arrhythmic and Cardiovascular Risk in End Stage Renal Disease (PACE) study. *BMC Nephrol*. 2015;16:63.
15. Fitzpatrick J, Sozio SM, Jaar BG, et al. Frailty, body composition and the risk of mortality in incident hemodialysis patients: the Predictors of Arrhythmic and Cardiovascular Risk in End Stage Renal Disease study. *Nephrol Dial Transplant*. 2019;34:346–354.
16. Patterson N, Price AL, Reich D. Population structure and eigenanalysis. *PLoS Genet*. 2006;2:e190.
17. Price AL, Patterson NJ, Plenge RM, et al. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet*. 2006;38:904–909.
18. Agatston AS, Janowitz WR, Hildner FJ, et al. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol*. 1990;15:827–832.
19. Wexler L, Brundage B, Crouse J, et al. Coronary artery calcification: pathophysiology, epidemiology, imaging methods, and clinical implications. A statement for health professionals from the American Heart Association. Writing Group. *Circulation*. 1996;94:1175–1192.
20. Nadkarni GN, Galarneau G, Ellis SB, et al. Apolipoprotein L1 variants and blood pressure traits in African Americans. *J Am Coll Cardiol*. 2017;69:1564–1574.
21. Chen TK, Appel LJ, Grams ME, et al. APOL1 risk variants and cardiovascular disease: results from the AASK (African American Study of Kidney Disease and Hypertension). *Arterioscler Thromb Vasc Biol*. 2017;37:1765–1769.
22. Kasembeli AN, Duarte R, Ramsay M, et al. APOL1 risk variants are strongly associated with HIV-associated nephropathy in Black South Africans. *J Am Soc Nephrol*. 2015;26:2882–2890.
23. Foster MC, Coresh J, Fornage M, et al. APOL1 variants associate with increased risk of CKD among African Americans. *J Am Soc Nephrol*. 2013;24:1484–1491.
24. Kanji Z, Powe CE, Wenger JB, et al. Genetic variation in APOL1 associates with younger age at hemodialysis initiation. *J Am Soc Nephrol*. 2011;22:2091–2097.
25. Tzur S, Rosset S, Skorecki K, Wasser WG. APOL1 allelic variants are associated with lower age of dialysis initiation and thereby increased dialysis vintage in African and Hispanic Americans with non-diabetic end-stage kidney disease. *Nephrol Dial Transplant*. 2012;27:1498–1505.
26. Chen TK, Katz R, Estrella MM, et al. Association between APOL1 genotypes and risk of cardiovascular disease in MESA (Multi-Ethnic Study of Atherosclerosis). *J Am Heart Assoc*. 2017;6:e007199.
27. Gutierrez OM, Limou S, Lin F, et al. APOL1 nephropathy risk variants do not associate with subclinical atherosclerosis or left ventricular mass in middle-aged black adults. *Kidney Int*. 2018;93:727–732.
28. Freedman BI, Langefeld CD, Lu L, et al. APOL1 associations with nephropathy, atherosclerosis, and all-cause mortality in African Americans with type 2 diabetes. *Kidney Int*. 2015;87:176–181.
29. Ma L, Shelness GS, Snipes JA, et al. Localization of APOL1 protein and mRNA in the human kidney: nondiseased tissue, primary cells, and immortalized cell lines. *J Am Soc Nephrol*. 2015;26:339–348.
30. Madhavan SM, O'Toole JF, Konieczkowski M, et al. APOL1 localization in normal kidney and nondiabetic kidney disease. *J Am Soc Nephrol*. 2011;22:2119–2128.