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APOL1 Renal-Risk Variants Do Not Associate With Incident Cardiovascular Disease or Mortality in the Systolic Blood Pressure Intervention Trial

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Introduction: Relationships between apolipoprotein L1 gene (*APOL1*) renal-risk variants (RRVs) and cardiovascular disease (CVD) remain controversial. To clarify associations between *APOL1* and CVD, a total of 2568 African American Systolic Blood Pressure Intervention Trial (SPRINT) participants were assessed for the incidence of CVD events (primary composite including nonfatal myocardial infarction, acute coronary syndrome not resulting in myocardial infarction, nonfatal stroke, nonfatal acute decompensated heart failure, and CVD death), renal outcomes, and all-cause mortality.

Methods: Cox proportional hazards regression models were used, adjusting for age, sex, African ancestry proportion, and treatment group (systolic blood pressure target of $<$120 mm Hg vs. $<$140 mm Hg).

Results: Of the participants, 14% had 2 *APOL1* RRVs; these individuals also had lower baseline estimated GFR and higher levels of albuminuria and BMI. After a median follow-up of 39 months, no significant association was observed between *APOL1* RRVs and the primary composite CVD outcome, any of its components, or all-cause mortality (recessive or additive genetic models). *APOL1* demonstrated a trend toward association with sustained 30% reduction in estimated GFR to $<$60 ml/min/1.73 m^2 in those with normal kidney function at baseline (hazard ratio 1.64; 95% confidence interval = 0.85−2.93; $P = 0.114$, recessive model).

Discussion: *APOL1* RRVs were not associated with incident CVD in high-risk hypertensive, nondiabetic African American participants in SPRINT.

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Apolipoprotein L1 gene (*APOL1*) G1 and G2 renal-risk variants (RRVs) are powerfully associated with a spectrum of progressive nondiabetic forms of nephropathy in individuals who possess recent African ancestry.[1,2] These primary kidney diseases reside in the focal segmental glomerulosclerosis spectrum and contribute to approximately 40% of end-stage kidney disease in African Americans.[3,4] *APOL1* is expressed in

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podocytes, glomerular endothelial cells, and renal tubular cells.[5,6] Several lines of evidence support intrinsic kidney *APOL1* gene expression and not circulating APOL1 protein as underlying the development of kidney disease.[7–11] *APOL1* expression is increased by interferons and other inflammatory mediators[12]; these factors may be the second hits required to cause kidney disease.[13] Postulated mechanisms whereby *APOL1* may cause kidney disease include APOL1 RRV proteins damaging cell membranes with loss of intracellular potassium and secondary activation of stress-activated protein kinases and mitochondrial dysfunction even prior to intracellular potassium depletion.[14,15]

In addition to the kidney, *APOL1* is expressed in the vasculature and its RRVs associate with high-density lipoprotein cholesterol particle concentrations.[5,6,16] Therefore, *APOL1* could be involved in the susceptibility to (or protection from) cardiovascular disease (CVD). Three studies have detected increased risk for CVD in individuals with 2 *APOL1* RRVs; however, paradoxically lower levels of subclinical CVD (based on coronary artery calcium) were detected, and these results could have been confounded by *APOL1* association with chronic kidney disease (CKD), a known contributor to CVD.[17,18] In contrast, several studies have reported protective effects of *APOL1* renal-risk variants on subclinical atherosclerosis, cerebrovascular disease, and all-cause mortality, and other studies saw no relationship between *APOL1* with CVD or survival.[19–24]

Potential therapeutic targets for preventing nephropathy include the *APOL1* gene and its protein products. Therefore, it is critical to determine whether *APOL1* RRVs protect from CVD, because inhibiting this gene to prevent kidney disease could accelerate atherosclerosis. The present analyses assessed relationships between *APOL1* RRVs with incident CVD outcomes, incident renal outcomes, and mortality in African Americans participating in the Systolic Blood Pressure Intervention Trial (SPRINT).

MATERIALS AND METHODS

Participants and Genotyping

SPRINT is a multicenter, randomized clinical trial of blood pressure control in individuals $\geq$ 50 years old at increased risk for CVD.[25] The high risk of CVD in SPRINT was based on Framingham Risk Score, prior CVD events, age $\geq$ 75 years, or CKD. Details of the intervention and outcomes have previously been reported.[26] Exclusion criteria included participants taking medications for diabetes mellitus at any time in the 12 months prior to baseline, urine albumin $>$ 600 mg/d or urine protein levels $>$ 1000 mg/d, or an eGFR $<$ 25 ml/min/1.73 m^2. Participants were randomized to systolic blood pressure control targets of $<$140 mm Hg (standard treatment) versus $<$120 mm Hg (intensive treatment).

Outcomes

The primary study outcome was a composite of CVD events; all were adjudicated, and they included myocardial infarction (MI), non-MI acute coronary syndrome, stroke, heart failure, and CVD death. A predefined participant subgroup included CKD, defined as eGFR $<$ 60 ml/min per 1.73 m^2 based on the 4-variable Modification of Diet in Renal Disease (MDRD) equation. Secondary outcomes in the CKD subgroup included the rate of development of ESRD and a 50% decline from baseline eGFR. Secondary outcomes in the non-CKD subgroup included the rate of ESRD and a 30% decrease from baseline eGFR with an end value $<$ 60 ml/min per 1.73 m^2. It should be noted that, because of when SPRINT was designed, the CKD subgroup and renal outcomes definitions were based on the MDRD eGFR equation. However, in the present analysis, all eGFRs are based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[27] Spot urine samples were collected for albumin and creatinine concentration to compute the urine albumin:creatinine ratio (UACR) at specified study visits. Incident albuminuria was defined as a doubling of UACR from $<$ 10 mg/g to $\geq$ 10 mg/g, confirmed by a subsequent laboratory test $\geq$ 90 days later.

Participants were recruited from approximately 100 clinics in the United States, and each had institutional review board approval. Written informed consent was obtained from all participants. The present analyses were limited to self-reported African American participants; of all 2802 African American SPRINT participants, 2568 (91.6%) consented to participation in genetics studies. Genotyping methods for *APOL1*, ancestry informative markers, and results of quality control have been reported.[20]

Statistical Analyses

The incidence of CVD events and all-cause mortality was estimated using standard Kaplan–Meier techniques, with log-rank tests to compare the incidence of these events by *APOL1* genotype within each treatment group. The time to first occurrence of CVD outcomes, all-cause mortality, and renal outcomes were compared by *APOL1* risk genotype using Cox proportional hazards regression models. Follow-up time was censored as of the National Heart, Lung, and Blood Institute Director decision to stop the

SPRINT intervention on 20 August 2015. We considered both an additive (0, 1, or 2 *APOL1* RRVs) and recessive (2 *APOL1* RRVs vs. 0 or 1) coding of *APOL1* risk genotype. All models included age, sex, proportion of African ancestry, and treatment group as covariates. Baseline eGFR and log(UACR) were also included as covariates for the CVD outcomes and all-cause mortality. We used multiple imputation (100 datasets) to address the small amount of missing data with baseline eGFR (n = 7, 0.3%) and UACR (n = 91, 3.5%). The imputation models included age, sex, smoking status, history of cardiovascular disease, systolic and diastolic blood pressure, use of angiotensin-converting enzyme inhibitors, use of angiotensin receptor blockers, body mass index, total cholesterol, high-densisty lipoprotein cholesterol, and log triglycerides as predictors. In addition, eGFR was included as a predictor in the imputation model for log(UACR).

The power to detect an association between being a carrier of 2 *APOL1* RRVs (recessive model) and incident events was estimated *a priori* assuming an α level of 0.05, 3 years of follow-up, and a loss to follow-up rate of 2% per year.[28] Assuming overall annual incidence rates of 0.5%, 1.0%, 1.5%, and 2.0% per year, we estimated that we would have at least 80% power provided that the hazard ratio associated with carrying 2 *APOL1* RRVs was at least 3.3, 2.3, 2.0, and 1.9, respectively.

Linear mixed-effect models were used to compare longitudinal trajectories for eGFR by *APOL1* risk genotype and treatment group, assuming an unstructured covariance matrix. For each combination of *APOL1* genotype and treatment group, we assumed a 2-slope linear model with a change-point at ≤6 months or >6 months postrandomization. The change-point in the slope of eGFR was designed to reflect mean trajectories in the acute phase of the intervention that were potentially due to hemodynamic effects (≤6 mo postrandomization) versus trajectories in the chronic phase (>6 mo postrandomization). Slopes for eGFR were compared using Wald tests based on the estimated model coefficients and SEs. Baseline eGFR, age, sex, history of CVD, and proportion of African ancestry were included in the model as covariates. Finally, because small (but statistically significant) changes in mean eGFR were observed in SPRINT when comparing fasting to nonfasting study visits, we included indicators denoting fasting visits as covariates, assuming separate effects for each of the 4 combinations of *APOL1* risk genotype and treatment group. All analyses were performed using SAS v9.4 (SAS Institute, Inc., Cary, NC) or the R Statistical Computing Environment (R Foundation for Statistical Computing; Vienna, Austria).

RESULTS

A total of 2568 African American SPRINT participants were included; 360 (14.0%) had 2 *APOL1* RRVs and 2208 (86%) had <2 RRVs. Table 1 displays baseline demographic and laboratory characteristics of individuals based on *APOL1* high-risk genotype. Participants with 2 *APOL1* RRVs had significantly higher body mass index, higher UACR, and lower eGFR but were otherwise similar to those with <2 *APOL1* RRVs. A significant (cross-sectional) association between

Table 1. Baseline demographic and laboratory data for African American participants with *APOL1* genotyping in Systolic Blood Pressure Intervention Trial (SPRINT)

Variable	APOL1 G1+G2 2 risk variants n = 360	APOL1 G1+G2 0/1 risk variants n = 2208	*P* value
Randomized to intensive treatment	190 (52.8)	1,074 (48.6)	0.162
Age (yr), mean ± SD	63.5 ± 9.0	64.4 ± 9.0	0.053
Female sex	169 (46.9)	997 (45.2)	0.565
Education			0.105
Less than high school	66 (18.3)	320 (14.5)	
High school	228 (63.3)	1,372 (62.1)	
College graduate	46 (12.8)	332 (15.0)	
Graduate degree	20 (5.6)	183 (8.3)	
Missing	0 (0.0)	1 (0.0)	
Alcohol consumption			0.663
Nondrinker	200 (55.6)	1,203 (54.5)	
Light drinker	72 (20.0)	415 (18.8)	
Moderate drinker	42 (11.7)	308 (13.9)	
Heavy drinker	22 (6.1)	156 (7.1)	
Missing	24 (6.7)	126 (5.7)	
Smoking status			0.167
Never smoker	169 (46.9)	950 (43.1)	
Former smoker	105 (29.2)	754 (34.2)	
Current smoker	86 (23.9)	501 (22.7)	
Pack-yr in smokers, mean ± SD	16.1 ± 18.9	16.2 ± 17.5	0.214
Body mass index (kg/m^2), mean ± SD	31.5 ± 6.7	30.8 ± 6.3	0.044
History of CVD	60 (16.7)	359 (16.3)	0.907
Systolic BP (mm Hg), mean ± SD	139.4 ± 15.8	139.8 ± 16.4	0.653
Diastolic BP (mm Hg), mean ± SD	82.3 ± 12.6	81.1 ± 12.4	0.081
eGFR (ml/min/1.73 m^2), mean ± SD	73.3 ± 24.6	76.8 ± 22.6	0.008
eGFR < 60 ml/min/1.73 m^2	102 (28.4)	524 (23.8)	0.069
UACR (mg/g), median (IQR)	12.2 (6.5–32.8)	8.7 (5.0–21.5)	<0.001
Albuminuria			<0.001
UACR ≤ 30 mg/g	255 (72.9)	1,707 (80.3)	
UACR >30 to ≤ 300 mg/g	71 (20.3)	354 (16.6)	
UACR > 300 mg/g	24 (6.9)	66 (3.1)	
Fasting glucose (mg/dl), mean ± SD	96.8 ± 13.9	97.9 ± 16.0	0.213
Total cholesterol (mg/dl), mean ± SD	195.8 ± 42.3	196.2 ± 40.6	0.837
HDL cholesterol (mg/dl), mean ± SD	54.4 ± 13.8	55.3 ± 15.2	0.280
No. of antihypertensive medications, mean ± SD	2.1 ± 1.1	2.0 ± 1.0	0.062
ACEi/ARB use	202 (56.1)	1,151 (52.1)	0.178
Statin use	123 (34.3)	744 (33.9)	0.944

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BP, blood pressure; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate, based on Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation; HDL, high-density lipoprotein; IQR, interquartile range; UACR, urine albumin:creatinine ratio. Data in parentheses reflect %, unless otherwise noted. Other rows display mean ± SD or median (IQR).

APOL1 RRVs with baseline UACR, serum creatinine concentration, and eGFR in SPRINT have previously been reported (recessive model).[20]

Kaplan–Meier curves by treatment group and *APOL1* risk genotype for the primary composite CVD outcome (nonfatal MI, acute coronary syndrome not resulting in MI, stroke, acute decompensated heart failure, and death from CVD) and the primary CVD composite plus all-cause mortality are shown in Figure 1 and Figure 2, respectively. Within each treatment group, there were no significant differences by *APOL1* genotype for either outcome (all $P > 0.15$). Table 2 displays hazard ratios (HRs) for the composite CVD outcome, its component events, all-cause mortality, and renal outcomes. After a median follow-up of 39.0 months (interquartile range = 33.8–45.5 mo), there were 22 adjudicated CVD events in the 360 participants with 2 *APOL1* RRVs versus 106 in the 2208 participants with < 2 *APOL1* RRVs (HR = 1.20, 95% confidence interval [CI] = 0.76–1.92, $P = 0.435$ recessive model; HR = 1.10, 95% CI = 0.86–1.41, $P = 0.458$ additive model). The HR for all-cause mortality, MI, and all CVD events comprising the primary SPRINT CVD outcome did not differ based on *APOL1* RRVs in either the additive or recessive model.

In contrast to significant relationships between *APOL1* RRVs and baseline (prevalent) kidney disease,[20] no significant *APOL1* associations were seen with the prespecified renal outcomes of: (i) 50% reduction in eGFR (measured twice ≥90 days apart), initiation of dialysis, or kidney transplantation; or (ii) proteinuria assessed as doubling of UACR from < 10 to ≥ 10 mg/g (measured twice ≥90 days apart in participants with CKD and baseline UACR < 10 mg/g) in the CKD subgroup (those with an initial eGFR < 60 ml/min per 1.73 m^2). In the subgroup without CKD at baseline, a nonsignificant trend was observed for the prespecified end-point of a 30% reduction in eGFR (measured twice ≥90 days apart) to an eGFR < 60 ml/min per 1.73 m^2, initiation of dialysis, or kidney transplantation (HR = 1.64, 95% CI = 0.85–2.93, $P = 0.114$ recessive model), but not for incident proteinuria.

Table 3 displays the slopes for eGFR decline using linear mixed models over the course of follow-up in African American SPRINT participants by treatment group and *APOL1* risk genotypes. Graphical depictions of eGFR group means over time and time estimated slopes from the mixed model analyses are presented in Supplementary Figure S1 (entire cohort), Supplementary Figure S2 (eGFR < 60 ml/min/1.73 m^2 at randomization), and Supplementary Figure S3 (eGFR ≥ 60 ml/min per 1.73 m^2 at randomization). Whether assessing eGFR slope during the initial 6 months following randomization or after 6 months, *APOL1* risk genotypes did not

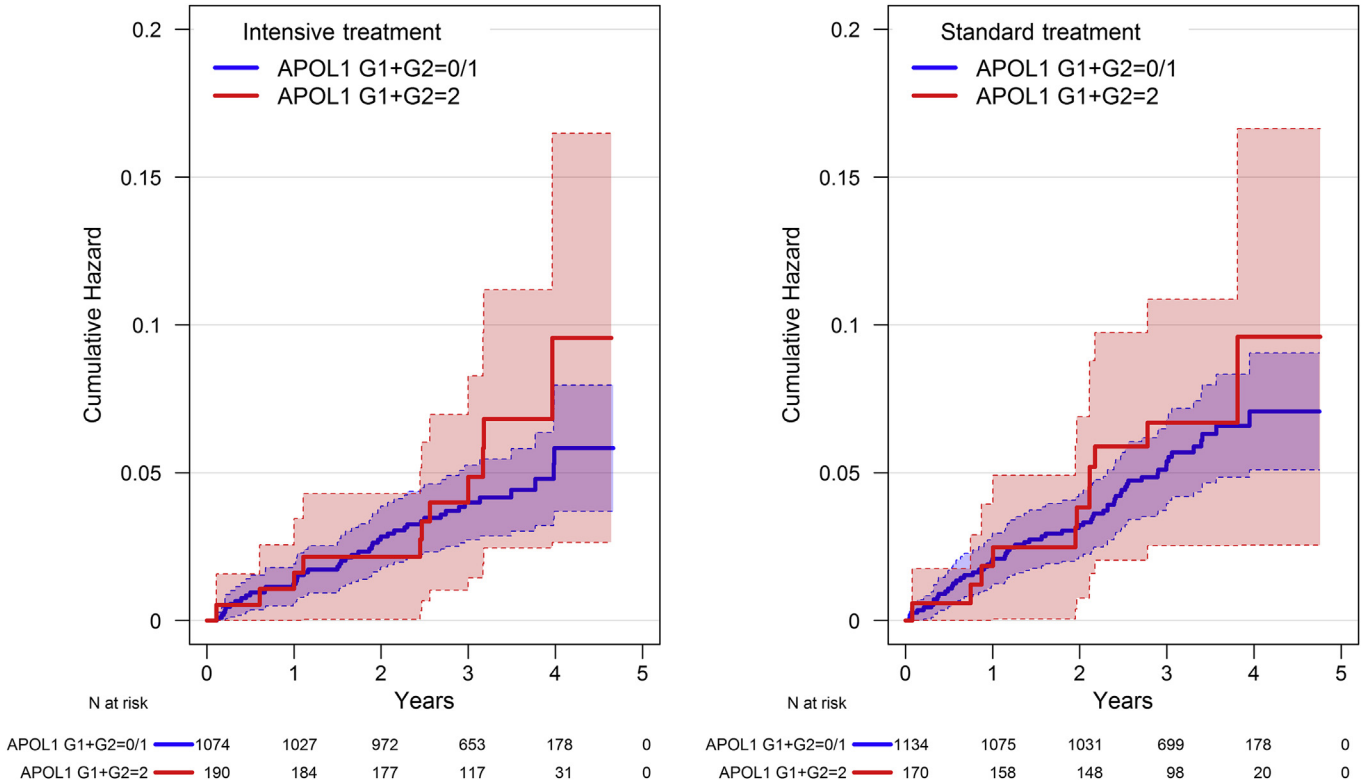

Figure 1. Kaplan–Meier curves for the primary cardiovascular disease (CVD) outcome in Systolic Blood Pressure Intervention Trial (SPRINT) for African American participants by treatment group and *APOL1* risk genotype.

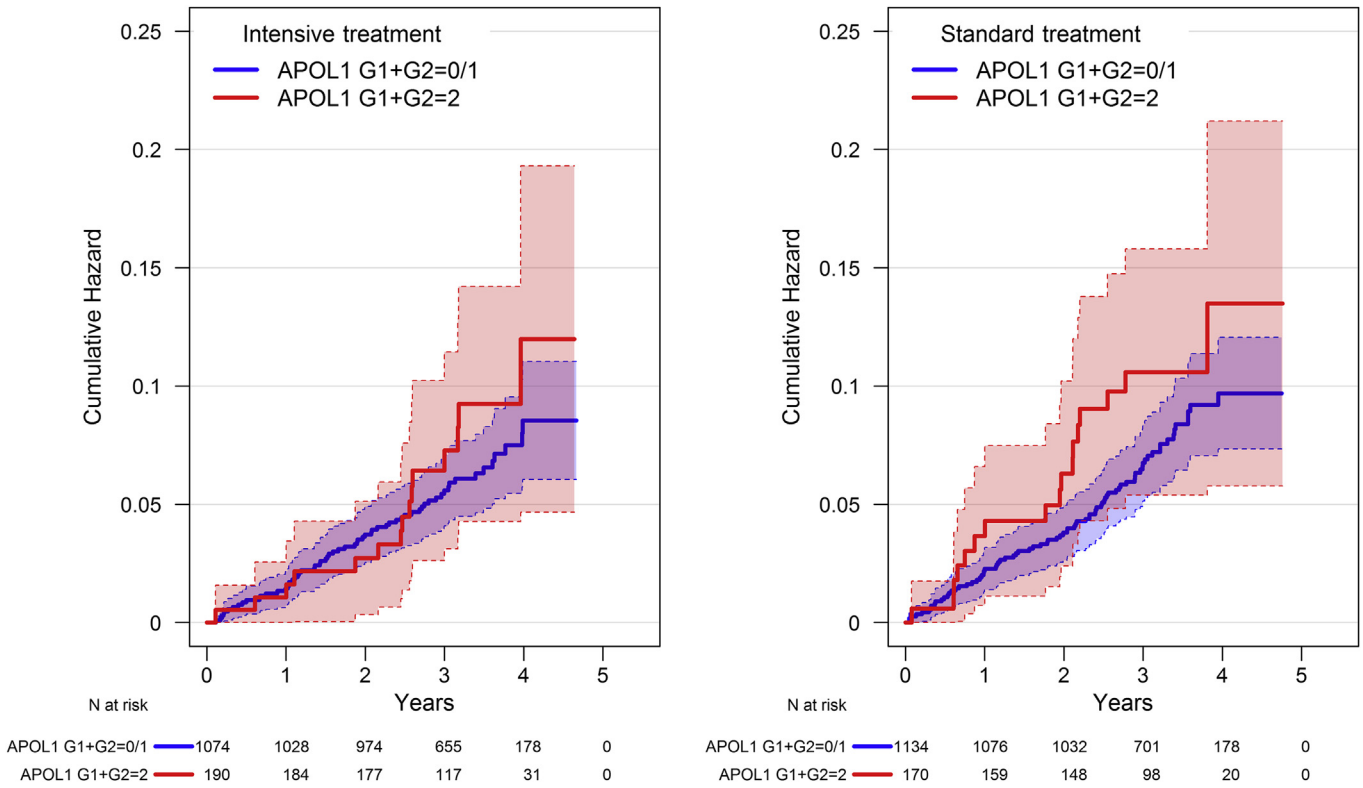

Figure 2. Kaplan–Meier curves for the primary cardiovascular disease (CVD) outcome plus all-cause mortality in Systolic Blood Pressure Intervention Trial (SPRINT) for African American participants by treatment group and *APOL1* risk genotype.

Table 2. Incidence of CVD, renal, and mortality outcomes by *APOL1* renal-risk genotype in African American SPRINT participants

	APOL1 G1+G2 = 2		*APOL1* G1+G2 = 0/1		Recessive model		Additive model	
	No. with events n = 360	% With events per yr (95% CI)	No. with events n = 2208	% With events per yr (95% CI)	HR (95% CI)	*P* value	HR (95% CI)	*P* value
All participants								
CVD primary outcome[a]	22	1.97 (1.30, 2.99)	106	1.53 (1.26, 1.85)	1.20 (0.76, 1.92)	0.435	1.10 (0.86, 1.41)	0.458
MI	6	0.53 (0.24, 1.18)	39	0.56 (0.41, 0.76)	1.02 (0.43, 2.45)	0.961	1.12 (0.73, 1.72)	0.607
ACS not resulting in MI	2	0.18 (0.04, 0.70)	12	0.17 (0.10, 0.30)	1.04 (0.23, 4.79)	0.958	1.02 (0.47, 2.20)	0.957
Stroke	7	0.62 (0.30, 1.30)	23	0.33 (0.22, 0.49)	1.66 (0.70, 3.92)	0.251	1.26 (0.77, 2.08)	0.359
Heart failure	7	0.62 (0.30, 1.30)	39	0.55 (0.41, 0.76)	0.98 (0.43, 2.23)	0.970	0.83 (0.54, 1.28)	0.403
CVD death	4	0.35 (0.13, 0.94)	22	0.31 (0.20, 0.47)	0.94 (0.32, 2.75)	0.905	1.27 (0.74, 2.16)	0.384
Nonfatal MI	6	0.53 (0.24, 1.18)	38	0.54 (0.39, 0.74)	1.07 (0.45, 2.57)	0.879	1.12 (0.72, 1.72)	0.618
Nonfatal stroke	7	0.62 (0.30, 1.30)	22	0.31 (0.21, 0.48)	1.76 (0.74, 4.18)	0.202	1.26 (0.76, 2.09)	0.379
Nonfatal heart failure	7	0.62 (0.30, 1.30)	37	0.53 (0.38, 0.73)	1.05 (0.46, 2.39)	0.905	0.89 (0.58, 1.38)	0.609
All-cause mortality	16	1.40 (0.86, 2.29)	79	1.11 (0.89, 1.39)	1.19 (0.69, 2.05)	0.530	1.10 (0.82, 1.46)	0.529
Primary + all-cause mortality	32	2.85 (2.02, 4.04)	145	2.09 (1.77, 2.46)	1.28 (0.87, 1.89)	0.212	1.10 (0.89, 1.36)	0.361
eGFR< 60 ml/min/1.73 m^2								
Primary CKD outcome[b]	1 / 102	0.29 (0.04, 2.09)	10 / 524	0.59 (0.32, 1.09)	0.48 (0.03, 2.51)	0.482	0.56 (0.19, 1.33)	0.228
Incident albuminuria[c]	0 / 21	—	23 / 201	3.87 (2.57, 5.83)	—	—	0.70 (0.35, 1.34)	0.301
eGFR ≥ 60 ml/min/1.73 m^2								
Secondary CKD outcome[d]	13 / 257	1.66 (0.97, 2.86)	53 / 1,678	0.99 (0.76, 1.29)	1.64 (0.85, 2.93)	0.114	1.06 (0.73, 1.50)	0.766
Incident albuminuria[c]	10 / 128	2.73 (1.47, 5.07)	61 / 961	2.08 (1.62, 2.67)	1.32 (0.63, 2.50)	0.417	1.41 (1.00, 1.99)	0.051

ACS, acute coronary syndrome; CKD, chronic kidney disease; CI, confidence interval; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HR, hazard ratio; MI, myocardial infarction; SPRINT = Systolic Blood Pressure Intervention Trial.
HRs for CKD and albuminuria outcomes adjusted for age, sex, African admixture, and treatment group. HRs for CVD outcomes and all-cause mortality additionally adjusted for eGFR and log (urine albumin:creatinine ratio).
[a]Includes nonfatal MI, ACS not resulting in MI, nonfatal stroke, nonfatal acute decompensated heart failure, and cardiovascular disease death.
[b]Includes a 50% reduction in eGFR (measured twice ≥90 days apart), dialysis, or kidney transplantation.
[c]Applies only to participants with urine albumin:creatinine ratio <10 mg/g at baseline, and required a doubling from < 10 mg/g to ≥ 10 mg/g (measured twice ≥90 days apart).
[d]Includes a 30% reduction in eGFR (measured twice ≥90 days apart) to an eGFR < 60 ml/min/1.73 m^2, dialysis, or kidney transplantation.

Table 3. Slopes from linear mixed model for estimated glomerular filtration rate (eGFR) over the course of follow-up in African American SPRINT participants by treatment group and *APOL1* risk genotype

Group	Time period[a]	Treatment group	*APOL1* G1+G2=2 Slope (95% CI)[b]	*APOL1* G1+G2=0/1 Slope (95% CI)[b]	*APOL1* G1+G2: 2 − 0/1 Difference (95% CI)	*P* value
All	Acute (≤6 mo)	Standard	−0.31 (−2.87, 2.26)	0.80 (−0.46, 2.07)	−1.11 (−3.69, 1.47)	0.398
All	Acute (≤6 mo)	Intensive	−4.18 (−6.57, −1.79)	−3.53 (−4.82, −2.25)	−0.65 (−3.06, 1.77)	0.600
All	Chronic (>6 mo)	Standard	−1.13 (−2.34, 0.08)	−0.97 (−1.42, −0.52)	−0.16 (−1.45, 1.12)	0.806
All	Chronic (>6 mo)	Intensive	−1.36 (−2.46, −0.26)	−1.01 (−1.47, −0.54)	−0.35 (−1.54, 0.84)	0.564
CKD (eGFR < 60 ml/min/1.73 m^2)	Acute (≤6 mo)	Standard	0.85 (−2.22, 3.91)	2.07 (0.48, 3.67)	−1.23 (−4.46, 2.00)	0.457
CKD (eGFR < 60 ml/min/1.73 m^2)	Acute (≤6 mo)	Intensive	0.06 (−2.93, 3.05)	−1.97 (−3.49, −0.45)	2.03 (−1.10, 5.16)	0.204
CKD (eGFR < 60 ml/min/1.73 m^2)	Chronic (>6 mo)	Standard	−1.41 (−2.50, −0.32)	−0.60 (−1.13, −0.06)	−0.81 (−2.03, 0.40)	0.190
CKD (eGFR < 60 ml/min/1.73 m^2)	Chronic (>6 mo)	Intensive	−0.82 (−1.91, 0.27)	−0.75 (−1.24, −0.26)	−0.07 (−1.26, 1.12)	0.908
Non-CKD (eGFR ≥ 60 ml/min/1.73 m^2)	Acute (≤6 mo)	Standard	−0.18 (−3.29, 2.93)	0.48 (−0.98, 1.94)	−0.66 (−3.77, 2.45)	0.679
Non-CKD (eGFR ≥ 60 ml/min/1.73 m^2)	Acute (≤6 mo)	Intensive	−5.44 (−8.27, −2.60)	−3.99 (−5.49, −2.48)	−1.45 (−4.31, 1.42)	0.322
Non-CKD (eGFR ≥ 60 ml/min/1.73 m^2)	Chronic (>6 mo)	Standard	−1.17 (−2.71, 0.36)	−1.20 (−1.72, −0.69)	0.03 (−1.59, 1.64)	0.975
Non-CKD (eGFR ≥ 60 ml/min/1.73 m^2)	Chronic (>6 mo)	Intensive	−1.83 (−3.18, −0.49)	−1.20 (−1.75, −0.64)	−0.64 (−2.09, 0.81)	0.388

CI, confidence interval; CKI, chronic kidney disease; SPRINT, Systolic Blood Pressure Intervention Trial.
[a]For acute time period (≤6 mo postrandomization), slopes reflect mean change in eGFR (in ml/min/1.73 m^2) over 6 mo. For chronic time period (>6 mo postrandomization), slopes reflect mean change in eGFR per 1 yr.
[b]Adjusted for baseline eGFR, age, sex, history of cardiovascular disease, proportion of African admixture, and whether or not measurement occurred at a fasting study visit.

significantly affect the rate of decline in kidney function in the standard or intensive treatment groups.

DISCUSSION

The present report assessed *APOL1* RRV associations with incident CVD and mortality in 2568 hypertensive, nondiabetic, African American SPRINT participants. After a median follow-up of 39 months and 177 total CVD events and deaths, no significant association was observed between *APOL1* RRVs and all-cause mortality, incident nonfatal MI, acute coronary syndrome without MI, stroke, heart failure, or the primary SPRINT composite CVD outcome. There were advantages to performing these analyses in SPRINT. The sample was relatively large, and CKD was generally mild. This cohort was also at high risk for CVD based on age and prior CVD events, yet only 14% possessed 2 *APOL1* RRVs. This is similar to the 13% frequency of 2 *APOL1* RRVs in the general population and should limit confounding of CKD with CVD.[1] Small numbers of CVD events may have limited study power. As such, meta-analyses including several studies are needed to help clarify the CVD effects of *APOL1*.

SPRINT results are similar to those reported in the Atherosclerosis Risk In Communities (ARIC) and African American Study of Kidney Disease and Hypertension (AASK), in which no association between *APOL1* RRVs and survival or CVD events was observed.[23,24] However, SPRINT, AASK, and ARIC results contrast with higher rates of CVD with *APOL1* RRVs in the Jackson Heart Study (JHS), Women's Health Initiative (WHI), and Cardiovascular Health Study (CHS).[17,18] *APOL1* RRVs were associated with baseline CKD, incident CKD, CKD progression, or albuminuria in all of these studies, so confounding between CKD and CVD is not likely to fully explain the differences in results. Confounding should be least likely in SPRINT and CHS, in which mild kidney disease was present and *APOL1* RRVs were associated only with baseline CKD or UACR, not with incident reductions in eGFR.[18] There were relatively few kidney disease events in SPRINT participants, an effect that reduced power to detect associations with renal outcomes. A trend toward sustained 30% reductions in eGFR to < 60 ml/min/1.73 m^2, need for dialysis, or kidney transplantation was seen with *APOL1* (recessive model) in the non-CKD subgroup ($P = 0.11$) (Table 2). No association was seen between *APOL1* genotypes and all-cause mortality; 95 deaths occurred during study follow-up. Several other studies reported protective effects of *APOL1* RRVs on related outcomes, including improved survival in nondiabetic patients on hemodialysis and in African American–Diabetes Heart Study (AA-DHS) participants, less calcified atherosclerotic plaque in AA-DHS, and less cerebrovascular disease (larger gray matter and smaller white matter lesion volumes) in AA-DHS MIND and SPRINT MND.[19,21,22]

It remains important to determine whether *APOL1* RRVs are associated with CVD, because targeting *APOL1* G1 and G2 variants to treat CKD could influence CVD outcomes. If *APOL1* RRVs are protective against CVD, there is a risk that atherosclerotic complications might develop from targeting this gene. If *APOL1* RRVs are associated with risk for CVD, targeting them could simultaneously reduce CVD and CKD. *APOL1* relationships with incident CVD outcomes in SPRINT, AASK, and ARIC suggest that significant associations do not exist.[23,24] In contrast, JHS, WHI, and CHS reported positive relationships between *APOL1* RRVs and incident CVD.[17,18] It should be noted that JHS

compared only African Americans with 2 versus no *APOL1* RRVs; results in participants with a single RRV (or in additive models) were not reported.[17] Furthermore, 2 *APOL1* RRVs in JHS was associated with lower levels of coronary artery calcified plaque (less subclinical CVD).[17] This was a paradoxical observation, given the higher reported risk for CVD in this group.[29]

As in prior reports assessing the effects of *APOL1* on CVD, SPRINT has strengths and limitations. Strengths included adjudication of CVD outcomes, renal outcomes, and deaths by an expert panel. Despite the relatively large sample of African Americans in SPRINT, we observed relatively few CVD events and deaths during a median of 39 months of follow-up. We were adequately powered to detect only strong associations between *APOL1* RRVs and incident CVD. As such, our data do not preclude effects of the magnitude reported, for example, in CHS, which had longer follow-up and more events.

In conclusion, results in SPRINT add to the expanding literature assessing relationships between renal-risk variants in the *APOL1* nephropathy gene and incident cardiovascular outcomes. Significant relationships were not observed between *APOL1* RRVs and incident nonfatal MI, stroke, heart failure, non-MI acute coronary syndrome, or CVD-related death in African American SPRINT participants. Significant relationships were also not observed with the composite of these outcomes or with all-cause mortality. Given the limitations of the current study and the existing conflicting reports in the literature, additional research is required in this important area.

DISCLOSURE

Wake Forest University Health Sciences and BIF have filed for a patent related to *APOL1* genetic testing. BIF receives research support from Novartis Pharmaceuticals and is a consultant for AstraZeneca and Ionis Pharmaceuticals. All the other authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Figure S1. Estimated glomerular filtration rate (eGFR) over the course of follow-up in African American SPRINT participants by treatment group and *APOL1* risk genotype. X-axis denotes study visits in months. Points and error bars reflect mean for eGFR (CKD-EPI equation) at each study visit with associated 95% confidence intervals. Lines represent estimated slopes from linear mixed model with change-point at 6-month study visit, assuming all visits were nonfasting visits (12-, 24-, and 48-month study visits were planned fasting visits). Counts below x-axis denote number of participants measured for each combination of treatment group and *APOL1* risk genotype.

Figure S2. Estimated glomerular filtration rate (eGFR) over the course of follow-up in African American SPRINT participants with eGFR $<$ 60 ml/min/1.73 m^2 at randomization by treatment group and *APOL1* risk genotype. X-axis denotes study visits in months. Points and error bars reflect mean for eGFR (CKD-EPI equation) at each study visit with associated 95% confidence intervals. Lines represent estimated slopes from linear mixed model with change-point at 6-month study visit, assuming all visits were nonfasting visits (12-, 24-, and 48-month study visits

were planned fasting visits). Counts below x-axis denote number of participants measured for each combination of treatment group and *APOL1* risk genotype.

Figure S3. Estimated glomerular filtration rate (eGFR) over the course of follow-up in African American SPRINT participants with eGFR $\geq$ 60 ml/min per 1.73 m^2 at randomization by treatment group and *APOL1* risk genotype. X-axis denotes study visits in months. Points and error bars reflect mean for eGFR (CKD-EPI equation) at each study visit with associated 95% confidence intervals. Lines represent estimated slopes from linear mixed model with change-point at 6 month study visit, assuming all visits were nonfasting visits (12-, 24-, and 48-month study visits were planned fasting visits). Counts below x-axis denote number of participants measured for each combination of treatment group and *APOL1* risk genotype.

Supplementary material is linked to the online version of the paper at http://www.kireports.org.

REFERENCES

1. Genovese G, Friedman DJ, Ross MD, et al. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. *Science*. 2010;329:841–845.
2. 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.
3. Kruzel-Davila E, Wasser WG, Aviram S, Skorecki K. APOL1 nephropathy: from gene to mechanisms of kidney injury. *Nephrol Dial Transplant*. 2016;31:349–358.
4. Freedman BI, Cohen AH. Hypertension-attributed nephropathy: what's in a name? *Nat Rev Nephrol*. 2016;12:27–36.
5. 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.
6. 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.
7. Reeves-Daniel AM, Depalma JA, Bleyer AJ, et al. The APOL1 Gene and Allograft Survival after Kidney Transplantation. *Am J Transplant*. 2011;11:1025–1030.
8. Lee BT, Kumar V, Williams TA, et al. The APOL1 genotype of African American kidney transplant recipients does not impact 5-year allograft survival. *Am J Transplant*. 2012;12:1924–1928.
9. Freedman BI, Pastan SO, Israni AK, et al. APOL1 genotype and kidney transplantation outcomes from deceased African American donors. *Transplantation*. 2016;100:194–202.
10. Bruggeman LA, O'Toole JF, Ross MD, et al. Plasma apolipoprotein L1 levels do not correlate with CKD. *J Am Soc Nephrol*. 2014;25:634–644.
11. Weckerle A, Snipes JA, Cheng D, et al. Characterization of circulating APOL1 protein complexes in African Americans. *J Lipid Res*. 2016;57:120–130.
12. Nichols B, Jog P, Lee JH, et al. Innate immunity pathways regulate the nephropathy gene Apolipoprotein L1. *Kidney Int*. 2015;87:332–342.
13. Freedman BI, Skorecki K. Gene-gene and gene-environment interactions in apolipoprotein L1 gene-associated nephropathy. *Clin J Am Soc Nephrol*. 2014;9:2006–2013.
14. Olabisi OA, Zhang JY, VerPlank L, et al. APOL1 kidney disease risk variants cause cytotoxicity by depleting cellular potassium and inducing stress-activated protein kinases. *Proc Natl Acad Sci U S A*. 2016;113:830–837.
15. Ma L, Chou JW, Snipes JA, et al. APOL1 renal-risk variants induce mitochondrial dysfunction. *J Am Soc Nephrol*. 2017;28:1093–1105.
16. Gutierrez OM, Judd SE, Irvin MR, et al. APOL1 nephropathy risk variants are associated with altered high-density lipoprotein profiles in African Americans. *Nephrol Dial Transplant*. 2016;31:602–608.
17. 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.
18. 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.
19. 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.
20. Langefeld CD, Divers J, Pajewski NM, et al. Apolipoprotein L1 gene variants associate with prevalent kidney but not prevalent cardiovascular disease in the Systolic Blood Pressure Intervention Trial. *Kidney Int*. 2015;87:169–175.
21. 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.
22. Freedman BI, Gadegbeku CA, Bryan RN, et al. APOL1 renal-risk variants associate with reduced cerebral white matter lesion volume and increased gray matter volume. *Kidney Int*. 2016;90:440–449.
23. Grams ME, Rebholz CM, Chen Y, et al. Race, APOL1 risk, and eGFR decline in the general population. *J Am Soc Nephrol*. 2016;27:2842–2850.
24. Ku E, Lipkowitz MS, Appel LJ, et al. Strict blood pressure control and mortality risk by APOL1 genotype. *Kidney Int*. 2017;91:443–450.
25. Wright JT Jr., Williamson JD, Whelton PK, et al. A randomized trial of intensive versus standard blood-pressure control. *N Engl J Med*. 2015;373:2103–2116.
26. Ambrosius WT, Sink KM, Foy CG, et al. The design and rationale of a multicenter clinical trial comparing two strategies for control of systolic blood pressure: the Systolic Blood Pressure Intervention Trial (SPRINT). *Clin Trials*. 2014;11:532–546.
27. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150:604–612.
28. Hsieh FY, Lavori PW. Sample-size calculations for the Cox proportional hazards regression model with nonbinary covariates. *Control Clin Trials*. 2000;21:552–560.
29. Detrano R, Guerci AD, Carr JJ, et al. Coronary calcium as a predictor of coronary events in four racial or ethnic groups. *N Engl J Med*. 2008;358:1336–1345.