

APOL1 Risk Variants Independently Associated With Early Cardiovascular Disease Death



Michael D. Hughson², Wendy E. Hoy¹, Susan A. Mott¹, John F. Bertram⁴, Cheryl A. Winkler⁵ and Jeffrey B. Kopp³

¹Centre for Chronic Disease, The University of Queensland, Brisbane, Queensland, Australia; ²Department of Pathology, University of Mississippi Medical Center, Jackson, Mississippi, USA; ³Kidney Disease Section, National Institute of Diabetes and Digestive and Kidney Disease, National Institutes of Health, Bethesda, Maryland, USA; ⁴Cardiovascular Program Biomedicine Discovery Institute and Department of Anatomy and Developmental Biology, School of Biomedical Sciences, Monash University, Melbourne, Victoria, Australia; and ⁵Basic Research Laboratory, Center for Cancer Research, National Cancer Institute, Leidos Biomedical Inc., Frederick National Laboratory, Frederick, Maryland, USA

Introduction: The relationship of *APOL1* renal risk variants to cardiovascular disease (CVD) is controversial and was the subject of this investigation.

Methods: Age, cause of death, and nephrosclerosis (the latter defined by glomerulosclerosis) were analyzed in the autopsies of 162 African Americans and 136 whites genotyped for *APOL1* risk alleles.

Results: Sudden deaths represented >75% of CVD autopsies for both races and all-risk genotypes. The average ages of CVD deaths for African Americans with 1 and 2 *APOL1* risk alleles were, respectively, 7.0 years ($P = 0.02$) and 12.2 years ($P < 0.01$) younger than African Americans with 0 risk alleles and 8.7 years ($P = 0.01$) and 13.9 years ($P = 0.01$) younger than whites. Age differences were not significant between African Americans and whites with 0 risk alleles ($P = 0.61$). The younger CVD deaths of African Americans were associated with less severe glomerulosclerosis with 2 ($P = 0.01$), although not 1 ($P = 0.09$), compared with 0 *APOL1* risk alleles. Cardiomyopathy was found in 23% of African Americans with 1 and 2 risk alleles and significantly contributed to the lower age ($P = 0.01$). For non-CVD deaths, age differences were not seen by race ($P = 0.28$) or among African Americans by risk allele status ($P = 0.38$).

Conclusion: Carriage of 1 or 2 *APOL1* risk alleles in African Americans was associated with earlier age deaths due to coronary artery disease and cardiomyopathy. For 2 risk alleles, the early age was independent of nephrosclerosis.

Kidney Int Rep (2018) 3, 89–98; <http://dx.doi.org/10.1016/j.ekir.2017.08.007>

KEYWORDS: *APOL1*; cardiovascular disease; hypertension; nephrosclerosis; race

© 2017 International Society of Nephrology. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Two apolipoprotein L1 (*APOLI*) variant coding alleles, termed G1 and G2, have been identified as major risk factors for progressive chronic kidney disease (CKD) among African Americans.¹ However, the *APOLI* association with cardiovascular disease (CVD) has been inconsistent, with studies suggesting variously enhanced risk or protective effects.²

The *APOLI* variant alleles are present exclusively on African origin chromosomes, with frequencies among African Americans of 23% for G1 and 13% for

G2 variant alleles.³ African Americans have an approximately 3.5-fold greater risk of end-stage kidney disease than US whites. The risk falls primarily in the diagnostic categories of hypertensive nephrosclerosis and focal segmental glomerulosclerosis, and is believed to be largely a recessive trait that requires the inheritance of 2 risk alleles that can be either G1 and/or G2.^{1,4–6}

Death rates for cardiac disease are higher among African Americans than US whites by 30%.⁷ In the Jackson Heart Study, Ito *et al.*⁸ found a 2-fold greater risk for CVD among African Americans with 2 *APOLI* risk alleles compared with a reference group with no risk alleles. Similarly, the Cardiovascular Health Study of Americans who were aged 65 years or older found an increased risk of myocardial infarction (adjusted hazard ratio: 1.8; 95% confidence interval: 1.1–3.0) among African Americans with 2 *APOLI* risk

Correspondence: Michael D. Hughson, Department of Pathology, University of Mississippi Medical Center, 2500 North State Street, Jackson, Mississippi 39216, USA. E-mail: mhughson@bellsouth.net

Received 12 June 2017; revised 5 August 2017; accepted 14 August 2017; published online 22 August 2017

alleles compared with white Americans or African Americans with 0 or 1 risk alleles.⁹ Associations between CVD and *APOL1* variants are complicated by findings that suggest the increased risk does not have relationships to hypertension, coronary artery calcification, carotid artery thickness, elevated C-reactive protein, or lower high-density lipoprotein levels that usually predispose individuals to coronary events.^{8–10}

In contrast, findings from the African American Study of Kidney Disease and Hypertension (AASK) and the Systolic Blood Pressure Intervention (SPRINT) Trial, *APOL1* risk variants demonstrated increased rates of progression of CKD, but no relationship was found with CVD or overall mortality.^{11,12}

For >10 years, we analyzed autopsy kidneys from a cross section of the Jackson, Mississippi community, a population from which the Jackson Heart Study was recruited.^{13–16} The original goal was to explore relationships among birth weight, intrinsic glomerular number, and adult hypertension. Because we did not want any significant pathological loss of glomeruli to interfere with counting, only normal to mild or moderately arteriosclerotic kidneys were included in the collection. The exclusion of significant renal pathology should have minimized any role for *APOL1*-related CKD that influenced CVD; yet, the age of death of African Americans was significantly younger than whites, and approximately one-third of all deaths were attributed to a combination of cerebrovascular disease and CVD, as previously reported from the present cohort.^{13,14} The purpose of the present study was to specifically examine the relationship of CVD deaths to *APOL1* risk genotype. Because of the associations with CVD risk, obesity, hypertension, and hypertension-associated nephrosclerosis were considered potential mediating factors.

METHODS

Approval for the study was obtained from the Institutional Review Board of the University of Mississippi Medical Center and the Human Research Ethics Committee of Monash University, Victoria, Australia. Consent was provided by next of kin.

The study cohort consisted of autopsies performed between 1998 and 2005 at the University of Mississippi Medical Center, Jackson, Mississippi. Clinical and demographic information was obtained from University of Mississippi Medical Center records. Race was ascertained from a combination of medical records, reports from next-of-kin, and physical examinations. The collection was primarily for an investigation of glomerular numbers in subjects without known renal disease. Specimens were

retained, and renal morphometry and stereology was performed if kidneys were grossly normal or showed only mild to moderate arterionephrosclerosis. Kidneys of significantly unequal size, and scarred or contracted kidneys were specifically excluded, as were kidneys with histological diabetic changes. Kidneys accepted for study were analyzed morphometrically for percent intimal thickening of interlobular arteries (Itr), percent cortical fibrosis, and percent of globally sclerotic glomeruli. At Monash University, kidney samples were analyzed stereologically by the disector and/or fractionator method for glomerular number (N_{glom}). These procedures have previously been described in detail.^{15,16}

In accordance with departmental quality assurance policy, gross, microscopic, and clinical autopsy findings were presented at a weekly autopsy conference, and pathology diagnoses and causes of death were formulated for the final autopsy report. For the present study, diagnoses from autopsy reports were categorized as: (i) coronary artery disease (CAD), if patients died of myocardial infarction, sudden death, or congestive heart failure, and had coronary artery atherosclerosis; (ii) cardiac (not CAD), if subjects died a sudden cardiac death or from congestive heart failure, but CAD was not present or was mild; (iii) cerebrovascular, if autopsy showed cerebral infarction, or intracerebral or subarachnoid hemorrhage; (iv) central nervous system, not cerebrovascular disease; and (v) pulmonary embolus, if death occurred in the community, and pulmonary emboli was the only significant pathological finding. Hospitalized patients dying of pulmonary emboli with another underlying disease were classified according to the underlying disease (e.g., cancer). Other causes of death included (i) cancer; (ii) pulmonary, not pneumonia; (iii) infection, if community-acquired and not a complication of a hospitalized illness; (iv) ethanol abuse and/or liver disease; (v) drug overdose; (vi) AIDS, for any of the complications of AIDS; (vii) accident; (viii) homicide; (ix) suicide; and (x) unknown or undetermined.

DNA was isolated from formalin-fixed, paraffin-embedded tissue on which previous morphometry and stereology had been performed. *APOL1* risk alleles were genotyped using TaqMan assays (ABI, Foster City, California).¹⁷ The *APOL1* G1 allele was defined as the presence of the S342G variant (rs73885319A>G) with or without the I384M (rs60910145T>G) variant, and the G2 allele was defined as the deletion of N388Y389 (rs71785313 TTATAA/–). The entire autopsy cohort on which morphometry and stereology was performed consisted of 192 African Americans and 150 whites. Genotyping of renal tissue was successfully

performed on 188 African Americans and 144 whites, of whom 162 African Americans and 136 whites were aged 18 years or older and were the subjects of the present report.

Medical records were reviewed for a history of hypertension, and subjects were categorized as hypertensive on the basis of a combination of the following as previously described^{15,16}: clinical history of hypertension, consistently elevated mean arterial blood pressure ≥ 107 mm Hg, severity of intrarenal arteriosclerosis, increased heart weight, and increased left ventricular wall thickness. Clinical blood pressures were available for the present study, and mean arterial pressure was calculated for 117 African Americans and 68 whites. Hypertension or nonhypertension status was assigned for 152 African Americans and 133 whites.

For statistical analysis, clinical characteristics were grouped by race and by risk genotype as 0 risk alleles, 1 risk allele, and 2 *APOL1* risk alleles. Analyses of data were performed with Stata/IC 10.0 (StataCorp Statistical Software, StataCorp LP, College Station, Texas) or with SigmaStat 3.5 (Systat Software Inc., Richmond, California). Multiple group comparisons were tested using a Kruskal-Wallis analysis of variance on ranks with Dunn's *post hoc* pairwise tests. Two-way comparisons of continuous variables were performed by a *t*-test if variables passed normality and equal distribution tests (Kolmogorov-Smirnov with Lilliefors's correction, SigmaStat 3.5), and using Wilcoxon rank sum tests if they did not pass. Categorical variables were evaluated by χ^2 or Fisher's exact tests. Spearman's rank-order, 2-way correlations were used to test relationships among age, hypertension, and variables related to nephrosclerosis that were considered as possible predictors of age in multivariable analyses. Multivariable analyses used linear regressions with age as the dependent variable and ordinal logistic regressions with *APOL1* risk genotype differences as the dependent variable. Among African Americans, 2 models were used to test age and *APOL1* genotype relationships. Model 1 compared the combination of 0 and 1 risk alleles versus 2 risk alleles (a recessive model). Model 2 tested additive relationships in age by ordinal logistic regression with genotypes designated as -1 for 0 risk alleles, 0 for 1 risk allele, and +1 for 2 risk alleles. Linear regression plots were generated that demonstrated the relationships between age of death versus glomerulosclerosis and age of death versus body mass index. The regression models for multivariable linear analyses and those for linear plots were tested by a Shapiro-Wilk test for normality of residuals of fitted values for age (Stata/IC10) and visually by kernel density plots. Fisher's

2-sided exact tests were used to evaluate whether genotype frequencies were in Hardy-Weinberg equilibrium.

RESULTS

The study cohort consisted of 162 African Americans and 136 whites. Among African Americans, 30 (19%) had 2 *APOL1* risk alleles, 69 (43%) had 1 risk allele, and 63 (39%) had 0 risk alleles. Among whites, 3 (2%) had 1 risk allele. The allele frequencies were similar to those reported in a sample of middle-aged African Americans enrolled in the Atherosclerosis Risk in Community Study.¹⁷ Women had a slightly, but not significantly, higher proportion of *APOL1* risk alleles than expected, and more female African Americans than male African Americans had 2 risk alleles ($P = 0.02$).

Supplementary Table S1 provides the causes of death and the average age of subjects by race. For each category of death, whites tended to be somewhat older than African Americans, but the difference was significant only for CAD ($P = 0.03$). There was no significant racial difference in the age of cerebrovascular disease death ($P = 0.09$). Twice as many out-of-hospital pulmonary thromboembolic deaths occurred among African Americans ($n = 14$, 8.6%) as whites ($n = 6$, 4.4%), but the proportional difference ($P = 0.22$) and ages ($P = 0.77$) were not significant. Among the African American pulmonary emboli deaths, the distribution of *APOL1* risk alleles was not significantly different than the distribution of alleles among all African American autopsies ($P = 0.73$).

Table 1 lists 24 cardiac disease deaths that were not attributable to CAD. These included 1 African American and 2 white patients with arrhythmogenic right ventricular dysplasia (none with *APOL1* risk alleles). There were 13 cases classified as hypertrophic or dilated cardiomyopathies, with 11 of the 13 investigated by the coroner as sudden out-of-hospital deaths. Of the 13 subjects with cardiomyopathies, 10 were African American, with 3 having 2 *APOL1* risk alleles and 5 having 1 risk allele. Those with 2 *APOL1* risk alleles included a hypertensive, obese 24-year-old who died suddenly of hypertrophic cardiomyopathy after an otherwise uneventful childbirth. None of the cases of hypertrophic and dilated cardiomyopathy showed any features of hereditary hypertrophic cardiomyopathy and were considered idiopathic, with 5 cases possibly being hypertensive cardiomyopathies. The cardiomyopathies were added together with CAD to create the combined category of CVD. None of 8 congenital, rheumatic, or infective cardiomyopathies were included in the CVD category.

Table 1. Cardiomyopathies without coronary atherosclerosis by race and *APOL1* genotype

Patient	Age	Race	Sex	BMI (kg/m ²)	G1	G2	Risk alleles	HTN	Heart weight (g)	Coroner's case	Diagnosis
1	25	W	M	24	0	0	0	No	500	No	Congenital heart disease
2	16	W	M	23	0	0	0	No	NR	No	Mucopolysaccharidosis
3	42	W	M	28	0	0	0	No	NR	No	Bicuspid aortic valve, died in surgery
4	18	AA	M	22	0	0	0	No	725	No	Congenital heart disease
5	30	W	M	38	0	0	0	No	375	No	Endocarditis
6	37	W	F	21	0	0	0	No	290	Yes	Endocarditis, IV drug use
7	27	AA	F	45	0	0	0	No	440	Yes	Pericarditis/myocarditis (viral?)
8	26	AA	M	26	0	0	0	Yes	625	No	Chronic rheumatic valve disease
9	33	W	F	25	0	0	0	No	320	Yes	Arrhythmogenic right ventricular dysplasia
10	18	W	M	22	0	0	0	No	345	Yes	Arrhythmogenic right ventricular dysplasia
11	21	AA	F	26	0	0	0	No	300	Yes	Arrhythmogenic right ventricular dysplasia
12	55	W	F	31	0	0	0	Yes	525	No	Dilated cardiomyopathy, treated HTN
13	38	W	F	24	0	0	0	No	350	Yes	Dilated cardiomyopathy
14	28	W	F	25	0	0	0	No	450	Yes	Dilated cardiomyopathy
15	44	AA	F	32	0	0	0	Yes	360	Yes	Dilated cardiomyopathy, treated HTN
16	33	AA	F	50	0	1	1	No	800	Yes	Dilated cardiomyopathy
17	29	AA	M	30	1	1	2	No	760	Yes	Dilated cardiomyopathy
18	46	AA	M	31	0	0	0	Yes	590	Yes	Hypertrophic cardiomyopathy
19	28	AA	M	27	1	0	1	No	580	Yes	Hypertrophic cardiomyopathy
20	22	AA	M	34	1	0	1	No	450	Yes	Hypertrophic cardiomyopathy
21	37	AA	M	25	0	1	1	Yes	620	Yes	Hypertrophic cardiomyopathy
22	38	AA	M	47	0	1	1	Yes	640	Yes	Hypertrophic cardiomyopathy
23	20	AA	F	45	0	2	2	No	450	Yes	Hypertrophic cardiomyopathy
24	25	AA	F	33	1	1	2	Yes	500	No	Hypertrophic cardiomyopathy [†]

AA, African Americans; BMI, body mass index; G1, non-synonymous amino acid substitutions S342G and I384M; G2, deletion of amino acid residues N388 and Y389; HTN, hypertension; NR, not recorded; W, white.

[†]Sudden death after childbirth.

Table 2 shows a Spearman 2-way rank-order correlation among age, hypertension, percent glomerulosclerosis, percent Itr, percent area of cortical fibrosis, and single N_{glom} for all subjects. Itr, glomerulosclerosis, cortical fibrosis, hypertension, and age were all closely correlated, with glomerulosclerosis having the strongest association with age and reduction in N_{glom} . As a gauge of nephrosclerosis, the findings indicated that, in this autopsy cohort, glomerulosclerosis could be used in multivariable analysis as a single measure for nephrosclerosis and its possible contribution to glomerular loss.

Table 3 presents the features of the autopsy cohort grouped as CVD and non-CVD, compared by race and risk genotype. The number and ages of subjects with CAD and cardiomyopathy are listed under CVD. Pathological findings of diabetic nephropathy were exclusionary criteria, and only 7 African Americans and 2 whites were diabetic, all with hypertensive, nondiabetic kidney changes. African Americans with 0 *APOL1* risk alleles were more frequently hypertensive than whites with 0 risk alleles in the category of CVD ($P = 0.04$), but not non-CVD ($P = 0.19$).

There was no significant difference in the frequency of CVD events between African Americans and whites with 0 risk alleles or among African Americans by

APOL1 risk genotype. Figure 1 illustrates the distribution of ages of CVD deaths among the subjects by race; *APOL1* risk genotype with cardiomyopathies is designated by open squares. Figure 1 shows that all CVD deaths with 2 *APOL1* risk alleles occurred at younger than 48 years of age, with the earliest death at 20 years of age occurring in an obese woman with hypertrophic cardiomyopathy. Ages in each race and risk allele category passed normality and equal variance tests, and were analyzed by *t*-tests.

Table 2. Correlation coefficients and *P* values for all subjects, African American and white

Characteristic	Hypertension	Itr (%)	Glomerulosclerosis (%)	Cortical fibrosis (%)	N_{glom}
Age	0.369	0.548	0.620	0.476	-0.103
<i>P</i> value	<0.001	<0.001	<0.001	<0.001	0.08
Hypertension		0.514	0.395	0.424	-0.132
<i>P</i> value		<0.001	<0.001	<0.001	0.02
Itr			0.554	0.558	-0.094
<i>P</i> value			<0.001	<0.001	0.12
Glomerulosclerosis (%)				0.675	-0.208
<i>P</i> value				<0.001	0.001
Cortical fibrosis					-0.144
<i>P</i> value					0.01

Spearman's two-way correlations for age, hypertension, percent interlobular artery intimal thickening (Itr), percent global glomerulosclerosis, percent cortical fibrosis, and single kidney glomerular number (N_{glom}). Significant values are in bold.

Table 3. Cause of death and characteristics by *APOL1* risk genotype in African Americans and whites

Cause of death	White		African American		African American	
	0 Risk alleles	0 Risk alleles	<i>P</i> (race)	1 Risk allele	2 Risk alleles	<i>P</i> (1 vs. 2)
All CVD (<i>n</i> = events)	45 (34%)	18 (29%)	0.46	26 (38%)	9 (30%)	0.46
Age (yr)	49.7 ± 12.8	48.0 ± 8.2	0.55	41.0 ± 9.7	35.8 ± 8.8	0.16
CAD (<i>n</i>)	42 (32%)	16 (25%)	0.47	21 (30%)	6 (20%)	0.41
Age	50.4 ± 12.6	48.4 ± 8.7	0.57	43.2 ± .0	41.3 ± 2.4	0.63
Cardiomyopathy	3 (2%)	2 (3%)	0.92	5 (7%)	3 (10%)	0.95
Age	40.3 ± 13.7	45 ± 1.4	0.68	31.6 ± 6.7	24.7 ± 4.5	0.16
Male	29 (64%)	12 (67%)	0.87	19 (73%)	4 (44%)	0.27
BMI (kg/m ²)	30 (24–34)	31 (28–34)	0.27	26 (24–38)	26 (24–35)	0.91
Hypertension	25 (56%)	15 (83%)	0.04	18 (69%)	6 (67%)	0.89
Diabetes	1 (2%)	2 (11%)	0.19	0	0	–
Heart weight (g)	480 (376–575)	539 (420–590)	0.54	450 (353–550)	450 (390–525)	0.92
Coroner's case	35 (78%)	15 (83%)	0.62	22 (85%)	6 (67%)	0.37
Glomerulosclerosis (%)	1.9 (1.2–3.7)	2.5 (1.3–6.1)	0.43	1.9 (0.9–3.5)	1.0 (0.3–1.4)	0.10
N _{glom} (×10 ³)	900 (708–1124)	913 (747–1169)	0.69	979 (654–1187)	779 (704–1132)	0.79
Non-CVD						
<i>N</i>	88	45		43	21	
Age (yr)	41.9 ± 13.9	39.1 ± 14.3	0.28	41.8 ± 14.2	41.5 ± 17.1	0.95
Male	55 (63%)	24 (53%)	0.31	19 (44%)	6 (29%)	0.23
BMI	27 (23–34)	27 (24–33)	0.91	29 (25–34)	26 (22–30)	0.08
Hypertension	23 (26%)	17 (38%)	0.19	23 (53%)	8 (38%)	0.21
Diabetes	1 (1%)	1 (2%)	1.00	4 (9%)	0	–
Heart weight (g) ^a	370 (315–475)	408 (360–493)	0.13	400 (318–558)	340 (280–380)	0.04
Coroner's case	53 (60%)	22 (49%)	0.24	16 (37%)	11 (52%)	0.25
Glomerulosclerosis (%)	1.5 (0.6–2.9)	1.3 (0.5–4.0)	0.93	2.9 (0.8–6.8)	2.5 (0.8–5.2)	0.71
N _{glom} (×10 ³)	911 (714–1148)	875 (726–1082)	0.47	860 (734–1044)	925 (789–1080)	0.25

BMI, body mass index; HTN, hypertension; N_{glom}, glomerular number, single kidney (right).

Ages for race and cardiovascular disease (CVD), coronary artery disease (CAD), and cardiomyopathy status passed skewness and kurtosis tests for normality and are recorded as mean ± SD. Other continuous variables were significantly skewed and are recorded as median and interquartile range. Significant values are in bold.

^aBetween CVD and non-CVD, heart weights were significantly different for whites ($P < 0.001$) and for African Americans with 0 ($P = 0.01$) and 2 risk alleles ($P = 0.01$).

Compared with whites with 0 *APOL1* risk alleles, the average age of CVD deaths for African Americans was younger by 8.7 years for 1 risk allele ($P = 0.01$) and 13.9 years for 2 *APOL1* risk alleles ($P = 0.01$). The average age of CVD deaths for African Americans with 1 risk allele was 7.0 years younger ($P = 0.02$), and for 2 *APOL1* risk alleles, the average age was 12.2 years younger ($P < 0.01$) than for African Americans with 0 risk alleles. Between African Americans and whites with 0 risk alleles, there were no significant differences in the ages of having CVD ($P = 0.61$), CAD ($P = 0.47$), or cardiomyopathy ($P = 0.92$) deaths. Between African Americans with 1 or 2 *APOL1* risk alleles, differences were not significant in the ages of CVD ($P = 0.16$), CAD ($P = 0.41$), or cardiomyopathy ($P = 0.16$) deaths; although African Americans with 1 risk allele were on average somewhat older than subjects with 2 risk alleles. There were no significant differences in age of non-CVD deaths between races ($P = 0.61$) with 0 risk alleles or between African Americans with 1 or 2 risk alleles ($P = 0.95$).

For CVD deaths, there was no significant difference in heart weights by race or among African Americans by *APOL1* risk genotype. African Americans who died of CVD had less severe glomerulosclerosis, with 2

compared with 0 ($P = 0.01$) but not 2 compared with 1 ($P = 0.10$) *APOL1* risk alleles, and with no significant difference in glomerulosclerosis between 0 and 1 risk

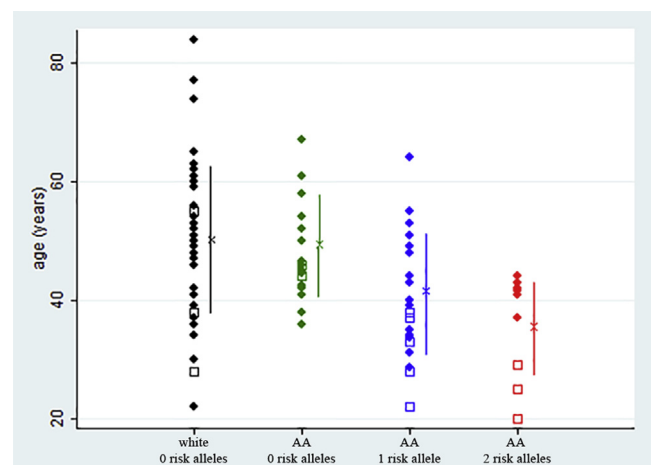


Figure 1. Age of cardiovascular disease (CVD) death versus race and *APOL1* risk genotype. The age of CVD death was significantly different between African Americans (AA) with 0 and 1 *APOL1* risk alleles ($P = 0.02$) and 0 and 2 *APOL1* risk alleles ($P < 0.01$). There was no significant difference between AA and whites with 0 risk alleles ($P = 0.61$) or between AA with 1 and 2 risk alleles ($P = 0.17$). Open symbols represent cardiomyopathies and solid symbols coronary artery disease.

alleles ($P = 0.21$). For CVD deaths, there was no significant differences in N_{glom} by *APOL1* genotype ($P = 0.82$). For non-CVD deaths, significant allelic differences in glomerulosclerosis and N_{glom} were not seen, but heart weight was significantly lower in African Americans with 2 *APOL1* risk alleles compared with 0 ($P = 0.001$) or 1 ($P = 0.04$) risk alleles.

Table 4 shows multivariable regression analyses for CVD, CAD, and cardiomyopathy. For race with 0 risk alleles and for model 1 of African Americans, the residuals of the fitted values for age of CVD and CAD deaths demonstrated statistically normal distributions. For cardiomyopathies, the residuals failed normality tests ($P < 0.001$).

By race, there were no significant differences among ages of CVD, CAD, or cardiomyopathy deaths in subjects with 0 *APOL1* risk alleles. In African American model 1 (recessive), the ages of CVD deaths of subjects with 2 risk alleles was significantly younger than subjects with 0 and 1 risk alleles (adjusted and unadjusted model), but age differences were not significant

for CAD or cardiomyopathy. In African American model 2 (additive), there was a significantly ordered decrease in age from 0 to 1 to 2 *APOL1* risk alleles in the categories of CVD (adjusted and unadjusted), CAD (unadjusted), and cardiomyopathy (adjusted and unadjusted).

Sex did not significantly influence the age of death, but glomerulosclerosis had a significant direct relationship with age of CVD and CAD deaths between races with 0 risk alleles and in model 1 for African Americans. The effect of glomerulosclerosis was significant for 0 and 1 risk alleles, and tended to diminish the influence of risk genotype on age of death. Figure 2 illustrates the direct relationships between glomerulosclerosis and older African American CVD deaths in subjects with 0 and 1 *APOL1* risk alleles, with deaths for each allele being observed at much older than 60 years of age. Figure 2 also shows that all CVD deaths with 2 risk alleles and more than one-half of CVD deaths in subjects with 1 risk allele occurred with only minimal glomerulosclerosis. Figure 3 shows the

Table 4. Multivariable analysis of age of cardiovascular (CVD), coronary artery disease (CAD) and cardiomyopathy deaths by clinical characteristics, glomerulosclerosis, and *APOL1* risk genotype

Cause of death	White versus African American		African American	
	0 n = 45 vs. n = 18 P, Coef (95% CI)	Model 1 recessive n = 44 vs. n = 9 P, Coef (95% CI)	Model 2 additive n = 18 > 26 > 9 P, Coef (95% CI)	
CVD				
Age	—	—	—	
Race	0.34, -3.0 (-9.2 to 3.2)	—	—	
Risk variants ^a	—	0.03, -8.1 (-15.1 to -0.5)	0.001, -0.1 (-0.2 to -0.04)	
Risk variants	—	0.04, -6.5 (-12.6 to -0.5)	0.01, -0.1 (-0.2 to -0.4)	
Sex	0.79, 0.8 (-5.0 to 6.6)	0.51, -1.6 (-6.6 to 3.3)	0.22, -0.8 (-2.0 to 0.5)	
BMI	0.49, -0.1 (-0.5 to 0.2)	0.01, -0.4 (-0.7 to -0.1)	0.03, -0.1 (-0.2 to -0.01)	
Glomerulosclerosis (%)	0.01, 0.7 (0.3 to 1.2)	<0.001, 0.8 (0.5 to 1.3)	0.74, -0.3 (-0.2 to 0.1)	
Model	R ² = 0.173, P = 0.02 SWilk test = 0.08	R ² = 0.426 P < 0.001 SWilk test = 0.17	pR ² = 0.165, P = 0.002	
CAD	n = 42 vs. n = 16	n = 37 vs. n = 6	n = 16 > 21 > 6	
Age	—	—	—	
Race	0.30, -3.4 (-10.0 to 3.1)	—	—	
Risk variants ^a	—	0.28, -4.1 (-11.7 to 3.5)	0.048, -0.1 (-0.1 to -0.00)	
Risk variants	—	0.41, -2.8 (-9.5 to 4.0)	0.10, -0.1 (-0.2 to 0.01)	
Sex	0.83, -0.7 (-6.9 to 5.6)	0.71, -0.9 (-6.0 to 4.1)	0.31, -0.7 (-2.1 to 0.7)	
BMI	0.30, -0.2 (-0.6 to 0.2)	0.10, -0.3 (-0.6 to 0.1)	0.02, -0.1 (-0.2 to -0.01)	
Glomerulosclerosis (%)	0.01, 0.7 (0.2 to 1.2)	<0.001, 0.8 (0.4 to 1.2)	0.50, -0.1 (-0.2 to 0.1)	
Model	R ² = 0.185, P = 0.03 SWilk test = 0.08	R ² = 0.372, P = 0.01 SWilk test = 0.13	pR ² = 0.137, P = 0.02	
Cardiomyopathy	n = 3 vs. n = 2	n = 7 vs. n = 3	n = 2 > 5 > 3	
Race	0.38, -8.9 (-84.7 to 66.8)	—	—	
Risk variants ^a	—	0.08, -10.8 (-23.2 to 1.5)	0.04, -0.4 (-0.8 to -0.02)	
Risk variants	—	0.28, -8.1 (-25.4 to 9.2)	0.03, -0.4 (-0.8 to -0.04)	
Sex	White all female	0.86, 1.3 (-17.1 to 19.7)	0.95, 0.1 (-3.8 to 4.1)	
BMI	0.18, 3.2 (-8.3 to 14.6)	0.87, -0.1 (-1.0 to 0.9)	0.86, 0.02 (-0.2 to 0.2)	
Glomerulosclerosis (%)	0.32, -38.2 (-37.7 to 28.2)	0.26, 4.2 (-4.3 to 12.6)	0.56, 0.8 (-1.9 to 3.5)	
Model	R ² = 0.943, P = 0.30 SWilk < 0.001	R ² = 0.523, P = 0.36 SWilk < 0.001	pR ² = 0.518, P = 0.03	

BMI, body mass index; CAD, coronary artery disease; CVD, cardiovascular disease.

Model, for multivariable adjustment. Shapiro Wilk (SWilk) test for normal distribution of residuals of fitted values for age. Significant values are in bold.

^aUnadjusted by multivariable analysis.

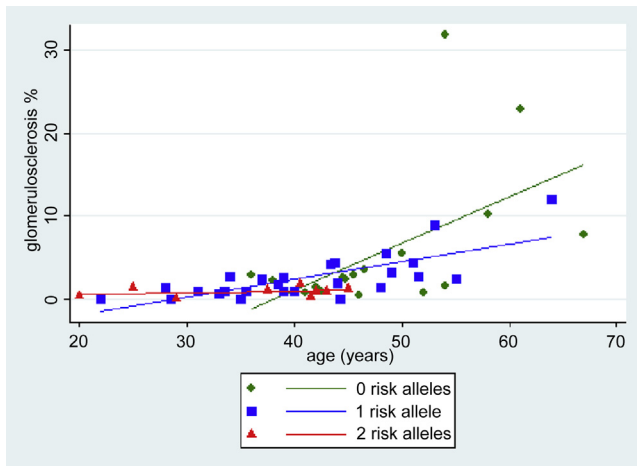


Figure 2. Age of cardiovascular disease death versus glomerulosclerosis among African Americans (AA) by *APOL1* risk genotype. Global glomerulosclerosis was related to age in AA with 0 and 1 *APOL1* risk alleles but not with 2 risk alleles (0 risk alleles, coefficient: 0.53; 95% confidence interval [CI]: 0.10–0.97; $P = 0.02$; 1 risk allele, coefficient: 2.52; 95% CI: 1.59–3.55; $P < 0.001$; 2 risk alleles, coefficient: 5.27; 95% CI: –6.86 to 17.4; $P = 0.34$). The slopes of the lines are different between 0 and 2 ($P = 0.02$) but not 0 and 1 ($P = 0.08$) and 1 and 2 ($P = 0.77$) *APOL1* risk alleles.

relationship between glomerulosclerosis and age of non-CVD deaths, in which for all risk genotypes, older age of death was associated with progressively severe glomerulosclerosis that began to increase at approximately 40 years of age.

Obesity had a significant inverse relationship with age of CVD death for African Americans with 1 ($P = 0.02$) and 2 ($P = 0.04$) risk alleles. **Figure 4**

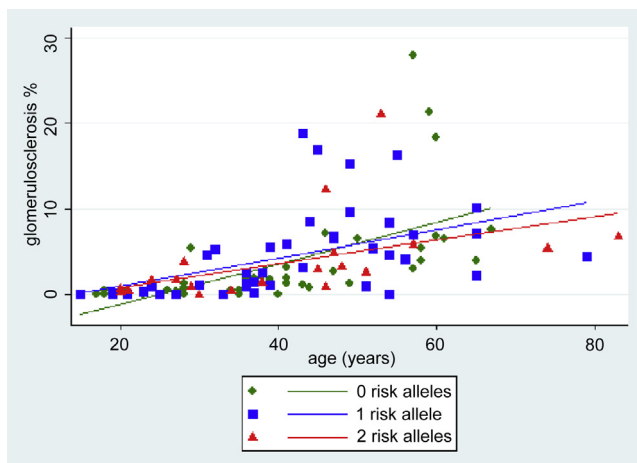


Figure 3. Age of non-cardiovascular disease death versus glomerulosclerosis among African Americans by *APOL1* risk genotype. For all risk allele groups, there was a significant relationship between age and extent of global glomerulosclerosis (0 risk alleles, coefficient: 1.51; 95% confidence interval [CI]: 0.89 to 2.14; $P < 0.001$; 1 risk allele, coefficient: 1.34; 95% CI: 0.54–2.15; $P < 0.01$; 2 risk alleles, coefficient: 1.66; 95% CI: 0.18–3.13; $P = 0.03$). There was no significant differences in the slopes of the regression lines.

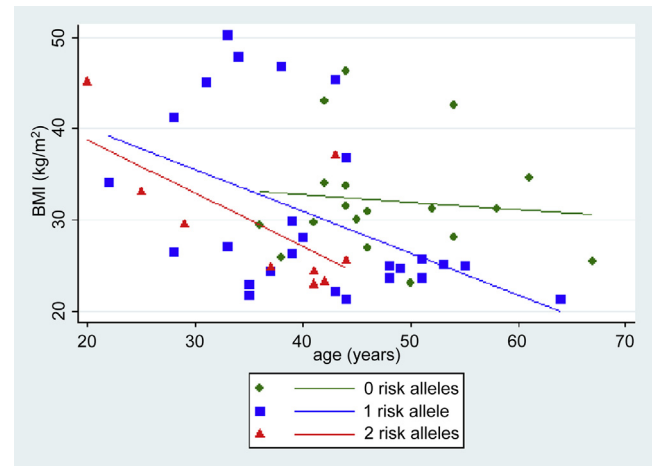


Figure 4. Age of cardiovascular disease (CVD) death versus body mass index (BMI) (kilograms divided by meters squared) among African Americans by *APOL1* risk genotype. Age at CVD death and BMI significantly correlated in subjects with 1 and 2 risk alleles (0 risk alleles, coefficient: –0.14; 95% confidence interval [CI]: –0.83 to 0.55; $P = 0.67$; 1 risk allele, coefficient: –0.47; 95% CI: –0.85 to –0.09; $P = 0.02$; 2 risk alleles, coefficient: –0.80; 95% CI: –1.56 to 0.04; $P = 0.04$). The slopes of the lines are significantly different between 0 and 1 ($P = 0.02$) and 0 and 2 ($P = 0.001$) but not 1 and 2 ($P = 0.09$) risk alleles.

illustrates these relationships and shows that most CVD deaths with 2 risk alleles were found among normal weight individuals. There was no significant relationship between obesity and age of non-CVD death (**Figure 5**). The residuals of the fitted values for age used in the linear regressions for all figures were normally distributed.

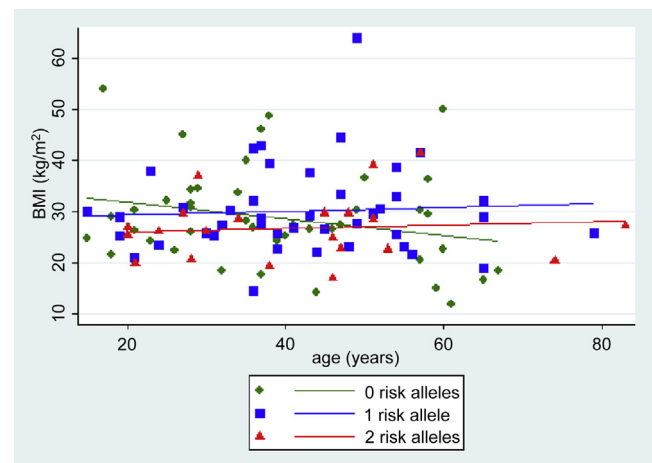


Figure 5. Age of non-cardiovascular disease (non-CVD) death versus body mass index (BMI) (kilograms divided by meters squared) among African Americans by *APOL1* risk genotype. There was no relationships between BMI and age of non-CVD death for any *APOL1* risk genotype (0 risk alleles, coefficient: –0.37; 95% confidence interval [CI]: –0.83 to 0.08; $P = 0.10$; 1 risk allele, coefficient: –0.11; 95% CI: –0.45 to 0.22; $P = 0.50$; 2 risk alleles, coefficient: 0.23; 95% CI: –1.04 to 1.50; $P = 0.71$). There were no significant differences in the slopes of the regression lines.

Cardiomyopathies accounted for 8 of 35 (23%) CVD deaths among African Americans with 1 or 2 risk alleles. The median age of these cardiomyopathy deaths was 40 years (interquartile range: 33–44 years), with all 8 cardiomyopathy deaths among subjects with 1 and 2 risk alleles occurring at younger than 39 years of age. For African Americans with 1 or 2 risk alleles, the contribution of cardiomyopathy to the distribution of the age of CVD death was estimated at 36% (Table 5).

DISCUSSION

This study population represented a cross section of the Jackson, Mississippi population selected by autopsy with >80% of CVD deaths being sudden and unexpected, which fell under the purview of the county coroner. Our study found that these CVD deaths occurred, on average, 8 years earlier in African Americans with 1 or 2 *APOL1* risk alleles compared with African Americans with 0 *APOL1* risk alleles, and occurred 10 years earlier than whites. The age of CVD death was not significantly different between whites and African Americans with 0 *APOL1* risk alleles, and *APOL1* genotype did not have any significant influence on the age of non-CVD deaths.

All CVD deaths in subjects with 2 risk alleles occurred at younger than 48 years of age without significant glomerulosclerosis. Although the severity of glomerulosclerosis predicted an older age of death for African Americans with 0 or 1 *APOL1* risk alleles, more than one-half of CVD deaths in subjects with 1 risk allele were associated with minimal glomerulosclerosis. The findings suggested that the effect of *APOL1* variants on early cardiac death might be independent of the factors promoting the enhanced nephrosclerosis associated with progressive renal disease.^{13,14} Most African Americans with death attributed to CVD were hypertensive. Nevertheless, among African Americans, the proportion of subjects with hypertension was not significantly different between different *APOL1* genotypes, which implied that a role for *APOL1* risk alleles in sudden cardiac deaths might be independent of blood pressure history.

The proportion of deaths attributed to CVD were not significantly different between whites and African

Americans or among African Americans by *APOL1* genotype, and were generally similar to the proportions of deaths attributed to CVD in most United States communities.⁷ Our sample was drawn from the same community participating in the longitudinal Jackson Heart study, in which Ito *et al.*⁸ identified African Americans with 2 *APOL1* risk alleles as being at a 2-fold greater risk for CVD than whites or African Americans with 0 *APOL1* risk alleles. Although we did not investigate CVD risk *per se*, the predominantly sudden in-community cardiac deaths represented in this study occurred at a notably younger age in subjects carrying 1 or 2 *APOL1* risk alleles. In addition, we noted that carriage of 1 or 2 *APOL1* risk alleles did not have any significant effect on age of out-of-hospital pulmonary thromboembolic deaths, a condition that might also disproportionately affect African Americans.¹⁸

In our study, the earlier cardiac deaths seen with a single *APOL1* risk allele raised the possibility of an additive or dominant characteristic. In the Cardiovascular Health Study of CVD risk after age 65 years, African Americans with 2 risk alleles had a 2-fold greater risk of incident myocardial infarction and died on average 3.4 years earlier than whites and 3.7 years earlier than African Americans with 0 or 1 risk alleles, which indicated a recessive trait.⁹ In our model 1, an earlier age of CVD death was seen when 2 *APOL1* risk alleles were compared with 0 and 1 risk alleles combined. This could be interpreted as recessive, but the younger age of CVD death was more strongly apparent when the ordered relationships of 0, 1, and 2 *APOL1* risk alleles were tested; this applied to both CAD and cardiomyopathy, which suggested that the cardiac effect of *APOL1* risk alleles might be additive.

It was difficult to reconcile these differences, but it should be noted that the increased CVD risk with 2 *APOL1* risk alleles was seen after 60 years of age in the Jackson Heart Study and after age 65 years in Cardiovascular Health Study.^{8,9} In the Jackson Heart Study, CVD deaths became more frequent in subjects with 2 *APOL1* risk alleles compared with those with 0 risk alleles between 50 and 55 years of age.⁸ This was slightly older than the average age of CVD death in our subjects with 0 *APOL1* risk alleles, but the average age was 8 years older than subjects with 1 and 2 *APOL1* risk alleles, which could indicate a partial, early effect of a single risk allele that might not continue with older age or that was masked by increased penetrance of 2 *APOL1* risk alleles with advancing age. In regard to the assignment of a gene effect, it should be recognized that carriage of 1 or 2 *APOL1* risk alleles does not, by itself, portend an early death. As Figures 3 and 5 illustrate, subjects with 1 or 2 risk alleles can live

Table 5. OR of CAD or cardiomyopathy versus age of CVD death among African Americans with 1 and 2 *APOL1* risk alleles

Disease	OR (95% CI)	P value	No.
CAD	1.33 (1.09–1.64)	0.01	27 of 35
Cardiomyopathy	0.75 (0.61–0.92)	0.01	8 of 35

CI, confidence interval.

The ages of coronary artery disease (CAD) and cardiomyopathy significantly contribute to the odds ratio (OR) of cardiovascular (CVD) death with the contribution of cardiomyopathy estimated at 36% ($0.75/2.08 = 0.36$).

into old age and die of conditions unrelated to CVD or kidney disease, an indication of the low penetrance that was reported for both conditions.^{8–11}

A novel feature of the present study was that it was autopsy-based and expanded the types of cardiac disease being evaluated beyond CAD. Subjects with cardiac deaths without coronary atherosclerosis included dilated and hypertrophic cardiomyopathy in 10 African Americans, among whom 5 were hypertensive, 7 were obese, and 3 of these 7 were massively obese. The autopsies of CVD subjects showed no significant differences in heart weight by race or by risk genotype. However, non-CVD subjects with 2 *APOL1* risk alleles had significantly smaller hearts than African Americans of similar age and hypertension status with 0 or 1 risk alleles. This seemed to differ from the findings of Ito et al.,⁸ who observed no imaging differences in cardiac hypertrophy or left ventricular geometry associated with *APOL1* risk alleles after 40 years of age, and suggested that young adults might be included in imaging studies that evaluate potential *APOL1* cardiac changes.

Mississippi residents have the highest rates of obesity in the United States,¹⁹ and among these autopsy subjects, both African Americans and whites had an average body mass index that indicated obesity, with many being severely obese. Although there were no significant racial or risk allele differences in body mass index, in the regression analyses shown in Figure 4, obesity was significantly related to earlier CVD deaths when 1 and 2 *APOL1* risk alleles were compared to 0 risk alleles. This probably did not implicate obesity as having a specific role in *APOL1*-related CVD deaths, because 6 of the 9 CVD deaths of subjects with 2 risk alleles occurred in nonobese individuals.

The major limitations of this study were the cross-sectional design, the small number of CVD subjects with 2 risk alleles, and the low statistical power. Nevertheless, in the Cardiovascular Health Study,⁹ the hazard ratio of a reduced median survival attributable to CVD among African Americans with 2 *APOL1* risk alleles compared with low risk African Americans and whites was 1.3 (95% confidence interval: 1.0–1.7; $P = 0.03$), which was a level of probability similar to that found for differences in age of CVD deaths in our autopsy study. The strength of our study was that it evaluated, in detail, the pathology of CVD in subjects with relatively mild hypertension-related renal disease. This was an important consideration because advanced hypertensive and any diabetic renal changes were exclusionary criteria, and potential relationships among CKD, diabetes, and CVD death were minimized.

APOL1 may function as an inflammatory mediator related to autophagy and pyroptosis.²⁰ In the

Cardiovascular Health Study, the higher CVD risk among African Americans with 2 risk alleles was associated with lower indexes of atherosclerosis.⁹ This implied a relationship to CAD that might be different than the inflammatory atherosclerotic plaque instability that commonly precedes coronary events.²¹ In the present study, a group of cardiomyopathies, that we considered idiopathic, included 23% of CVD deaths among African Americans with 1 and 2 *APOL1* risk alleles. The variable findings suggested that the *APOL1* genotype might have a distinctive influence on cardiac pathophysiology and lead to diverse phenotypes with the underlying mechanisms, because as with kidney disease, these have yet to be clearly defined.^{3,5,21}

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

The study was supported by a project grant from the National Health and Medical Research Council (NHMRC) of Australia (#194276), by an untied grant from the Colonial Foundation of Australia (Hoy, 2001–2011), an NHMRC Australian Fellowship (#511081, Hoy), by the National Institutes of Health (NIH) National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) (R01-DK065970-01), and a grant from the American Heart Association, Southeastern Affiliate (2001–2003). This work was supported in part by the Intramural Research Program of the NIDDK and National Cancer Institute by federal funds from NIH, under contract HHSN26120080001.

SUPPLEMENTARY MATERIAL

Table S1. Causes of death with the number of subjects and average age by race.

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

REFERENCES

1. Freedman BI, Skoreki K. Gene-gene and gene-environment interactions in apolipoprotein L1 gene-associated nephropathy. *Clin J Am Soc Nephrol*. 2014;9:2006–2013.
2. McLean N, Robinson TW, Freedman BI. *APOL1* gene kidney risk variants and cardiovascular disease: getting to the heart of the matter. *Am J Kidney Dis*. 2017;70:281–289.
3. Genovese G, Friedman DJ, Ross MD, et al. Association of trypanolytic *APOL1* variants of kidney disease in African Americans. *Science*. 2010;329:841–845.
4. Freedman BI, Kopp JB, Langefeld CD, et al. The apolipoprotein L1 (*APOL1*) gene and non-diabetic nephropathy in African Americans. *J Am Soc Nephrol*. 2010;21:1422–1426.
5. Parsa A, Kao WH, Xie D, et al. AASK Study Investigators; CRIC Study Investigators. *APOL1* risk variants, race, and

- progression of chronic kidney disease. *N Engl J Med.* 2013;369:2183–2196.
6. 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.
 7. Mensah GA, Mokdad AH, Ford ES, Greenlund KJ, Croft JB. State of disparities in cardiovascular health in the United States. *Circulation.* 2005;111:1233–1241.
 8. 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.
 9. 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.
 10. Freedman BI, Kopp JB, Langefeld CD, et al. The apolipoprotein L1 (APOL1) gene and nondiabetic nephropathy in African Americans. *J Am Soc Nephrol.* 2010;21:1422–1426.
 11. Langefeld CD, Divers J, Pajewski NM, et al. Systolic Blood Pressure Intervention Trial (SPRINT). 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.
 12. Freedman BI, Rocco MV, Bates JT, et al, for the SPRINT Research Group. APOL1 renal-risk variants do not associate with incident cardiovascular disease or mortality in the Systolic Blood Pressure Intervention Trial. *Kidney Int Rep.* 2017;2:713–720.
 13. Hoy WE, Hughson MD, Kopp JB, Mott SA, Bertram JF, Winkler CA. APOL1 risk alleles are associated with exaggerated age-related changes in glomerular number and volume in African-American adults: an autopsy study. *J Am Soc Nephrol.* 2015;26:3179–3189.
 14. Hughson MD, Hoy WE, Mott SA, et al. APOL1 risk alleles are associated with more severe arteriosclerosis in renal resistance vessels with aging and hypertension. *Kidney Int Rep.* 2016;1:10–23.
 15. Hughson MD, Gobe G, Douglas-Denton RN, Hoy WE, Bertram JF. Associations of glomerular number and birth weight with clinicopathological features of African Americans and whites. *Am J Kidney Dis.* 2008;52:18–28.
 16. Hughson MD, Puelles VG, Hoy WE, et al. Hypertension, glomerular hypertrophy and nephrosclerosis: the effect of race. *Nephrol Dial Transplant.* 2014;29:1399–1409.
 17. Atta MG, Estrella MM, Kuperman M, et al. HIV-associated nephropathy patients with and without apolipoprotein L1 gene variants have similar clinical and pathological characteristics. *Kidney Int.* 2012;82:338–343.
 18. Purav Mody P, Aakriti Gupta A, Bikkeli B, et al. Most important articles on cardiovascular disease among racial and ethnic minorities. *Circ Cardiovasc Qual Outcomes.* 2012;5:e33–e41.
 19. Robert Wood Johnson Foundation. The state of obesity: better policies for a healthier America. Mississippi state obesity data, rates and trends. Available at: <http://stateofobesity.org/states/ms>. Accessed March 18, 2017.
 20. Zhaorigetu S, Wan G, Kaini R, Jiang Z, Hu CA. APOL1, a BH3-only lipid binding protein, induces autophagic cell death. *Autophagy.* 2008;4:1079–1082.
 21. Farrall M. Cardiovascular twist to the rapidly evolving apolipoprotein L1 story. *Circ Res.* 2014;114:746–747.