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Original Investigation

APOL1 Genotype and Glomerular and Tubular Kidney Injury in Women With HIV

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Background: *APOL1* genotype is associated with advanced kidney disease in African Americans, but the pathogenic mechanisms are unclear. Here, associations of *APOL1* genotype with urine biomarkers of glomerular and tubular injury and kidney function decline were evaluated.

Study Design: Observational study.

Setting & Participants: 431 human immunodeficiency virus (HIV)-infected African American women enrolled in Women's Interagency HIV Study (WIHS).

Predictor: *APOL1* genotype.

Outcomes: Albumin-creatinine ratio (ACR), 4 tubular injury biomarkers (interleukin 18 [IL-18], kidney injury molecule 1 [KIM-1], neutrophil gelatinase-associated lipocalin [NGAL], and α_1-microglobulin [A1M]), and kidney function estimated using the CKD-EPI cystatin C equation.

Measurements: Participants were genotyped for *APOL1* single-nucleotide polymorphisms rs73885319 (G1 allele) and rs71785313 (G2 allele). Urine biomarkers were measured using stored samples from 1999-2000. Cystatin C was measured using serum collected at baseline and 4- and 8-year follow-ups.

Results: At baseline, ACRs were higher among 47 women with 2 *APOL1* risk alleles versus 384 women with 0/1 risk allele (median, 24 vs 11 mg/g; $P < 0.001$). Compared with women with 0/1 risk allele, women with 2 risk alleles had 104% higher ACRs (95% CI, 29-223 mg/g) and 2-fold greater risk of ACR > 30 (95% CI, 1.17-3.44) mg/g after multivariable adjustment. *APOL1* genotype showed little association with urine IL-18:Cr ratio, KIM-1:Cr ratio, and NGAL:Cr ratio (estimates of -5% [95% CI, -24% to 18%], -20% [95% CI, -36% to -1%], and 10% [95% CI, -26% to 64%], respectively) or detectable urine A1M (prevalence ratio, 1.13; 95% CI, 0.65-1.97) in adjusted analyses. Compared with women with 0/1 allele, women with 2 risk alleles had faster eGFR decline, by 1.2 (95% CI, 0.2 to 2.2) mL/min/1.73 m^2 per year, and 1.7- and 3.4-fold greater rates of incident chronic kidney disease (95% CI, 1.1 to 2.5) and 10% annual eGFR decline (95% CI, 1.7 to 6.7), respectively, with minimal attenuation after adjustment for glomerular and tubular injury biomarker levels.

Limitations: Results may not be generalizable to men.

Conclusions: Among HIV-infected African American women, *APOL1*-associated kidney injury appears to localize to the glomerulus, rather than the tubules.

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INDEX WORDS: *APOL1* genotype; risk variant; risk allele; G1 allele; G2 allele; single-nucleotide polymorphism (SNP); albumin-creatinine ratio (ACR); proteinuria; tubular injury biomarker; apolipoprotein L1; kidney disease; renal function; glomerular injury; African American; Women's Interagency HIV Study (WIHS).

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African Americans have a 3- to 4-fold greater risk of end-stage renal disease (ESRD) compared with whites in human immunodeficiency virus (HIV)-infected and uninfected populations.[1-4] Two prior studies revealed strong associations between chromosome 22q and ESRD among nondiabetic individuals of African ancestry.[5,6] Subsequent studies identified 2 risk variants on the *APOL1* (apolipoprotein L1) gene that account for these associations.[7,8] The G1 allele comprises 2 single-nucleotide polymorphisms (reference SNP identification numbers rs73885319 and rs60910145) encoding 2 amino acid substitutions and the G2 allele encodes a 2–amino acid deletion (rs71785313).[7] Heterozygosity for the risk alleles appears sufficient to confer resistance to *Trypanosoma brucei rhodesiense,*[9] whereas homozygosity or compound heterozygosity leads to substantially elevated kidney disease risk.[7]

However, the pathogenesis of APOL1-associated kidney disease is poorly understood. Madhavan et al[10] demonstrated that APOL1 localizes to podocytes, proximal tubules, and endothelium in the normal kidney and that glomerular and proximal tubular APOL1 staining is reduced among individuals with focal segmental glomerulosclerosis and HIV-associated nephropathy (HIVAN). Ma et al[11] subsequently reported that APOL1 protein and messenger RNA were detectable in podocytes and renal tubular cells of cryosections from individuals with normal kidney function. However, the specific effects of *APOL1* risk alleles on glomerular and proximal tubular function were not evaluated.

Biomarkers of tubular injury and dysfunction may be useful in earlier detection and localization of pathology within the nephron. In the Women's Interagency HIV Study (WIHS), we previously found that African American race was a strong and independent risk factor for albuminuria and was associated with higher levels of 3 urinary markers of tubular injury and dysfunction: interleukin 18 (IL-18), neutrophil gelatinase-associated lipocalin (NGAL), and α_1-microglobulin (A1M).[12,13] Furthermore, albuminuria, IL-18, and A1M levels were each independently associated with longitudinal kidney function decline and mortality.[13-15] These findings suggest that the higher risk of kidney disease progression among HIV-infected African Americans may be mediated through their more extensive glomerular and tubular injury. However, specific contributions of the *APOL1* risk alleles to these observations have not been ascertained.

In this study of HIV-infected African Americans enrolled in the WIHS, we first evaluated the cross-sectional associations of *APOL1* risk alleles with glomerular injury, quantified by albumin-creatinine ratio (ACR), as well as 4 biomarkers of tubular injury and dysfunction: IL-18, kidney injury molecule 1 (KIM-1), NGAL, and A1M. We then assessed the associations of *APOL1* risk alleles with longitudinal kidney function decline, adjusting for baseline levels of the injury markers.

METHODS

Study Population

The WIHS is a prospective cohort study that enrolled 3,067 HIV-infected and 1,070 uninfected women from 6 US locations (Bronx, Brooklyn, Chicago, Los Angeles, San Francisco, and Washington, DC) in 1994 to 1995, 2001 to 2002, or 2011 to 2012. Details of the study design and data collection methods were published previously.[16,17] Participants underwent semiannual visits that included a standardized questionnaire, physical examination, and collection of laboratory specimens. Among the 795 African American HIV-infected women who were evaluated at the WIHS clinical visits from 1999 to 2000, we chose a priori to include all 431 women who had the following: (1) consent for DNA testing, (2) available DNA for *APOL1* genotyping, and (3) stored urine available from the 1999 to 2000 visits for biomarker measurements.

The institutional review boards of participating institutions approved the study protocol at all WIHS study sites, and informed consent was obtained from all study participants. This cross-sectional study was also approved by the Institutional Review Board at the Johns Hopkins School of Medicine.

Exposure Variable

Participants were genotyped for the *APOL1* SNPs rs73885319 (G1 allele) and rs71785313 (G2 allele) using TaqMan assays (Applied Biosystems)[18] and categorized as having 0, 1, or 2 risk alleles. The G1 allele rs60910145 was not tested because rs73885319 and rs60910145 are in near-perfect linkage disequilibrium.[7] Genotyping for ancestry informative markers was performed using the Illumina GoldenGate platform. Selection of ancestry informative markers in the WIHS was described previously.[19] Briefly, West African, European, East Asian, and Native American populations were distinguished using a subset of markers published by Smith et al.[18] Of the larger panel of 384 ancestry informative markers, 185 were selected and 168 passed quality control measures. Genetic ancestry components were evaluated by principal components analysis using EIGENSTRAT software.[20,21]

Outcome Variables

All biomarkers were measured at the Cincinnati Children's Hospital Medical Center Biomarker Laboratory using random urine samples. Urine specimen storage and coefficients of variation for the biomarker assays are described in Item S1 (provided as online supplementary material). Urine albumin and creatinine were measured by immunoturbidimetry and colorimetric enzyme assay, respectively, using a Siemens Dimension Xpand plus HM clinical analyzer. Urine IL-18 was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Medical & Biological Laboratories Co). The urine KIM-1 ELISA was constructed using commercially available reagents (R&D Systems Inc).[22] Urine NGAL was assayed using a human-specific commercially available ELISA (BioPorto).[23] Urine A1M was measured by a commercially available assay (Siemens BN II Nephelometer).

Serum for cystatin C measurement was collected at baseline and 2 follow-up visits, occurring approximately 4 and 8 years after the initial visit. Kidney function was estimated using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation for serum cystatin C, which is less susceptible to bias by muscle mass and health status than serum creatinine.[24] Cystatin C was measured centrally at the University of California, Los Angeles Clinical

Immunology Research Laboratory using a particle-enhanced immunoturbidimetric assay (Gentian), which has been calibrated against the new World Standard Reference material ERM-DA471/IFCC (International Federation of Clinical Chemistry and Laboratory Medicine).[25] Additional details are described in Item S1.

Continuous outcomes included baseline and annual change in estimated glomerular filtration rate (eGFR) in milliliters per minute per 1.73 m^2 during approximately 8 years of follow-up. Additionally, 2 dichotomous kidney outcomes were analyzed: (1) incident chronic kidney disease (CKD), defined as eGFR $<$ 60 mL/min/1.73 m^2 at either of 2 follow-up visits among participants with baseline eGFRs $\geq$ 60 mL/min/1.73 m^2, and (2) rapid decline, defined as $\geq$10% annual decline in eGFR.

Covariates

Demographic and clinical characteristics were ascertained at the time of urine collection (1999-2000). Hypertension was defined by self-reported use of antihypertensive medications, systolic blood pressure $\geq$ 140 mm Hg, diastolic blood pressure $\geq$ 90 mm Hg, or self-reported diagnosis of hypertension confirmed by any of the previous criteria within 2 years. Diabetes mellitus was defined by self-reported use of oral hypoglycemic medications or insulin, fasting blood glucose level $\geq$ 126 mg/dL, or self-reported history of diabetes diagnosis confirmed by either of the former criteria within 2 years. Hepatitis C virus (HCV) coinfection was defined by detectable HCV RNA and positive HCV antibody result. HIV-related characteristics included current CD4 lymphocyte count, history of clinical AIDS diagnosis,[26] HIV RNA level, and current combination antiretroviral therapy (cART) use. Historical suppression of HIV RNA was defined as percentage of historical HIV RNA $<$ 400 copies/mL.

Statistical Analysis

We stratified women by number of *APOL1* risk alleles and compared demographic and clinical characteristics using χ^2 and Kruskal-Wallis tests for categorical and continuous variables, respectively. We then evaluated the associations of *APOL1* risk alleles with creatinine-standardized and -unstandardized albumin, IL-18, KIM-1, and NGAL levels using linear regression with robust Huber-White standard errors[27,28] (to correct for heteroscedasticity). Due to right-skewed distributions, continuous outcomes were log-transformed to normalize their distributions; results were back-transformed to produce estimated percentage differences.

Consistent with our previous WIHS publication,[13] A1M was handled as a dichotomous variable, detectable or undetectable. Because A1M is fully reabsorbed by proximal tubular cells after filtration at the glomerulus,[29] its presence in urine indicates proximal tubular dysfunction.[30] We used Poisson relative risk regression with a robust variance estimator[31] to assess the associations of *APOL1* risk alleles with detectable A1M and with the clinically established threshold for ACR $>$ 30 mg/g.

Primary analyses were performed using a recessive model of inheritance (2 vs 0/1 risk alleles). In secondary analyses, we evaluated dominant (2/1 vs 0 risk alleles) and additive (2 vs 1 vs 0 risk alleles) models of inheritance.

We then constructed multivariable regression models to determine whether *APOL1* risk genotype was independently associated with each biomarker. Model covariates were selected on the basis of known biological associations with the development and progression of kidney disease and included age, hypertension, diabetes mellitus, body mass index, HCV infection, HIV viral load, CD4 lymphocyte count, cART use, and baseline eGFR. Additionally, we adjusted for genetic ancestry data by including in our models the principal components that were associated with the biomarkers in univariate analyses (components 1, 3, and 5). We assessed for effect modification between *APOL1* risk genotype and historical suppression of HIV RNA for the outcome of albuminuria by evaluating an interaction term in the overall model. To determine whether APOL1-associated kidney injury precedes overt CKD, we also performed subgroup analyses restricted to individuals with eGFRs $\geq$ 60 mL/min/1.73 m^2.

Finally, we evaluated the associations of *APOL1* risk genotype with kidney function at baseline and during follow-up using a recessive model of inheritance. We used linear mixed models to evaluate the associations of *APOL1* risk alleles with annual eGFR change, with random intercepts and slopes across time to account for the correlation between repeated measures at baseline and years 4 and 8. We then used Poisson regression with a robust variance estimator to assess the associations of *APOL1* risk alleles with incident CKD and rapid decline, expressing results as incident rate ratios for each outcome. Women with eGFRs $<$ 60 mL/min/1.73 m^2 at baseline were excluded from incident CKD analyses. Multivariable models sequentially adjusted for demographics, traditional risk factors for kidney disease progression, ACR, and the tubular injury markers. To account for losses to follow-up in kidney decline analyses, we adjusted estimates using an inverse probability weighting approach by modeling the participant's probability of having a nonmissing outcome, with separate weights calculated at each visit.[32] The inverse of this probability was then used as a weight applied to persons with known outcomes in the multivariable regression analyses of kidney decline.

Multiple imputation with the Markov chain Monte Carlo method was used to impute missing covariates, with 10 imputations to yield $\sim$95% relative efficiency.[33] The percentage of missing observations for each covariate ranged from $<$1% to 31% (Table S1). Right-skewed variables were log-transformed in the imputation model. Estimates obtained from imputed data were combined using the MIANALYZE procedure to reflect the uncertainty due to missing data.

Compared with the women included in this substudy, women who were excluded (n = 364) due to missing *APOL1* genotype or urine biomarker measurements had a higher prevalence of diabetes mellitus, lower CD4 lymphocyte count, and lower prevalence of cART use (Table S2). As an alternate approach, we used multiple imputation to include all 795 HIV-infected African American women in the analyses (Tables S3 and S4).

RESULTS

Study Population by *APOL1* Genotype

Among the 431 HIV-infected African American women included in this study, 11% (n = 47) and 44% (n = 191) had 2 and 1 *APOL1* risk alleles, respectively, which are similar to frequencies reported for the general African American population in the United States.[34] Hypertension and HCV infection were each present in 31% and 35% of participants, respectively, whereas diabetes was present in 10% of the cohort. There were no statistically significant differences in prevalences of hypertension, diabetes mellitus, or HCV infection by number of *APOL1* risk alleles (Table 1). Median CD4 cell count and HIV RNA levels were similar across *APOL1* risk allele groups.

Associations of *APOL1* Genotype With Glomerular and Tubular Injury Markers

The distribution of ACRs among women with 2 *APOL1* risk alleles was shifted to the right relative to those with 0/1 risk allele, indicating higher ACRs

Table 1. Baseline Characteristics of HIV-Infected African American Women Stratified by Number of *APOL1* Risk Alleles

Characteristic	0 Allele (n = 193)	1 Allele (n = 191)	2 Alleles (n = 47)	*P*
Baseline age (y)	41 [35-46]	42 [37-47]	37 [35-47]	0.1
Hypertension	50 (26)	67 (35)	17 (36)	0.1
Diabetes mellitus	13 (7)	26 (14)	6 (13)	0.08
HCV infection	66 (35)	67 (35)	16 (34)	0.9
Current $CD4^+$ cell count (cells/μL)	380 [246-563]	388 [242-583]	474 [318-673]	0.09
History of AIDS	86 (45)	96 (50)	25 (53)	0.4
HIV RNA load				0.8
≤80 copies/mL	52 (28)	56 (29)	13 (28)	
81-1,999 copies/mL	42 (22)	47 (25)	10 (22)	
2,000-9,999 copies/mL	30 (16)	29 (15)	11 (24)	
≥10,000 copies/mL	65 (34)	59 (31)	12 (26)	
Historical HIV suppression[a]	68 (35)	73 (38)	15 (32)	0.7
Current cART use	117 (61)	116 (61)	23 (49)	0.3

Note: Values for categorical variables are given as number (percentage); values for continuous variables, as median [interquartile range]. *P* values from χ^2 and Kruskal-Wallis tests for categorical and continuous variables, respectively.

Abbreviations: cART, combination antiretroviral therapy; HCV, hepatitis C virus; HIV, human immunodeficiency virus.

[a]Defined as percentage of historical HIV RNA values < 400 copies/mL for each study participant.

(Fig 1A). By contrast, the distributions of IL-18, KIM-1, NGAL, and A1M did not appear to differ by number of *APOL1* risk alleles (Figs 1B and S1A-C).

Using a recessive model of inheritance (Table 2), the presence of 2 *APOL1* risk alleles compared to 0/1 risk allele was associated with higher ACRs (median, 24 vs 11 mg/g; $P < 0.001$) and doubling in the prevalence of ACR > 30 mg/g (47% vs 21%; $P = 0.001$). After multivariable adjustment for traditional kidney risk factors and HIV-related risk factors (Table 3), the presence of 2 *APOL1* risk alleles remained associated with 104% higher ACR ($P = 0.002$) and 2-fold greater prevalence of ACR > 30 mg/g ($P = 0.02$). There was no significant interaction between historical suppression of HIV RNA and *APOL1* genotype for the outcome of ACR ($P = 0.4$).

APOL1 genotype was associated with higher ACRs when analyzed using dominant ($P = 0.02$) and additive ($P < 0.001$) models of inheritance (Table 2). After multivariable adjustment, the observed associations remained robust using the additive ($P = 0.01$) but not the dominant ($P = 0.6$) model (Table 3).

APOL1 risk genotype was not associated with higher levels of urine IL-18, NGAL, or detectable A1M by recessive, dominant, or additive models of inheritance (Tables 2 and 3). In unadjusted analyses, *APOL1* risk genotype was associated with lower urine levels of creatinine-standardized but not -unstandardized KIM-1 in recessive and additive models. However, after multivariable adjustment (Table 3), the association between *APOL1* risk genotype and creatinine-standardized KIM-1 no longer met statistical significance. Urine

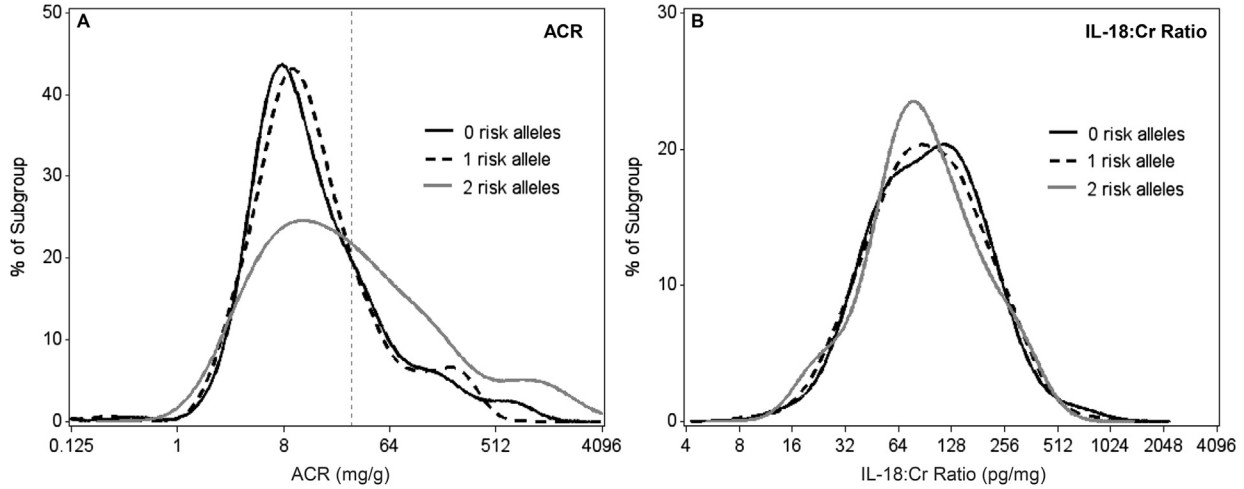

Figure 1. Distributions of (A) albumin-creatinine ratio (ACR) and (B) interleukin 18 to creatinine (IL-18:Cr) ratio in human immunodeficiency virus (HIV)-infected African American women, stratified by number of *APOL1* risk alleles. Dashed vertical line indicates ACR threshold of 30 mg/g.

Table 2. Urine Biomarker Levels in HIV-Infected African American Women Stratified by Number of *APOL1* Risk Alleles

				P		
	0 Allele (n = 193)	1 Allele (n = 191)	2 Alleles (n = 47)	Recessive[a]	Dominant[b]	Additive[c]
Glomerular injury						
Cr-standardized						
ACR (mg/g)	10 [7-29]	11 [7-24]	24 [8-88]	<0.001	0.02	<0.001
ACR > 30 mg/g	44 (23)	38 (20)	22 (47)	0.001	0.1	0.005
Unstandardized						
Urine albumin (mg/mL)	0.16 [0.08-0.37]	0.16 [0.09-0.37]	0.40 [0.11-1.10]	<0.001	0.009	<0.001
Tubular injury						
Cr-standardized						
IL-18:Cr ratio (pg/mg)	97 [55-149]	94 [56-150]	79 [59-148]	0.3	0.4	0.3
KIM-1:Cr ratio (pg/mg)	323 [228-493]	325 [219-568]	265 [171-465]	0.007	0.06	0.01
NGAL:Cr ratio (ng/mg)	29 [15-65]	34 [16-58]	27 [14-75]	0.9	0.7	0.9
Unstandardized						
IL-18 (pg/mL)	122 [66-247]	131 [70-253]	119 [83-215]	0.9	0.9	0.9
KIM-1 (pg/mL)	459 [241-874]	530 [310-866]	415 [252-845]	0.3	0.6	0.3
NGAL (ng/mL)	39 [19-86]	44 [21-91]	38 [21-159]	0.5	0.3	0.4
Tubular dysfunction						
Detectable A1M	118 (61)	130 (68)	30 (64)	0.7	0.8	0.9
Urine Cr (mg/mL)	1.50 [0.91-2.10]	1.54 [0.96-2.08]	1.51 [0.98-2.27]	0.5	0.4	0.4

Note: Values for categorical variables are given as number (percentage); values for continuous variables, as median [interquartile range].

Abbreviations: A1M, α_1-microglobulin; ACR, albumin-creatinine ratio; Cr, creatinine; HIV, human immunodeficiency virus; IL-18, interleukin 18; KIM-1, kidney injury molecule 1; NGAL, neutrophil gelatinase-associated lipocalin.

[a] *P* value for 2 versus 0/1 risk alleles, adjusted for age and ACR.

[b] *P* value for 2/1 versus 0 risk alleles, adjusted for age and ACR.

[c] *P* value for trend (2 vs 1 vs 0 risk alleles), adjusted for age and ACR.

creatinine levels were similar across *APOL1* risk allele groups (Table 2).

Findings were similar when we restricted analyses to participants with baseline eGFRs ≥ 60 mL/min/1.73 m^2 (n = 408; Tables S5 and S6) and when we used multiple imputation to include all participants with missing *APOL1* genotype or urine biomarker measurements (n = 795; Table S3).

Associations of *APOL1* Genotype With Longitudinal Kidney Function Decline

Mean baseline eGFR was 83.5 mL/min/1.73 m^2 in women with 2 *APOL1* risk alleles and 89.1 mL/min/1.73 m^2 in women with 0/1 risk allele ($P = 0.2$). During approximately 8 years of follow-up, women with 2 *APOL1* risk alleles experienced faster kidney function decline compared with women with 0/1 risk allele (Table 4). Unadjusted rates of annual eGFR decline were −2.6 (95% confidence interval [CI], −3.5 to −1.6) mL/min/1.73 m^2 among women with 2 risk alleles and −1.3 (95% CI, −1.6 to −1.1) mL/min/1.73 m^2 among women with 0/1 risk allele ($P = 0.02$). Adjustment for common risk factors, ACR, and the tubular injury marker levels minimally affected the magnitude of difference in annual kidney function decline by *APOL1* risk genotype.

During follow-up, 100 (25%) and 34 (8%) cases of incident CKD and rapid decline occurred, respectively. Compared with women with 0/1 risk allele, women with 2 *APOL1* risk alleles experienced higher rates of incident CKD and rapid decline by 1.7- and 3.4-fold, respectively, with minimal attenuation after adjustment for the kidney injury marker levels (Table 4).

Findings were similar when we performed longitudinal analyses in all 795 HIV-infected African American participants, using multiple imputation to include individuals with missing *APOL1* genotype or urine biomarker measurements (Table S4).

DISCUSSION

Among HIV-infected African American women, we found that the presence of 2 *APOL1* risk alleles is associated with albuminuria, a traditional marker of glomerular injury, but not with higher levels of tubular injury markers, including IL-18, KIM-1, NGAL, and A1M. In longitudinal analyses, women with 2 *APOL1* risk alleles experienced faster kidney function decline and higher rates of incident CKD and 10% annual eGFR decline compared with women having 0/1 risk allele. Notably, adjustment for baseline levels of glomerular and tubular injury markers did not attenuate the associations of *APOL1* risk

Table 3. Adjusted Associations of *APOL1* Risk Alleles With Biomarkers in HIV-Infected African American Women

	% Estimate[a] (95% CI)	Prevalence Ratio[b] (95% CI)	*P* by Model of Inheritance		
			Recessive[c]	Dominant[d]	Additive[e]
Continuous outcomes					
ACR (mg/g)	104 (29 to 223)	—	0.002	0.6	0.01
IL-18:Cr ratio (pg/mg)	−5 (−24 to 18)	—	0.6	0.5	0.5
KIM-1:Cr ratio (pg/mg)	−20 (−36 to −1)	—	0.06	0.5	0.06
NGAL:Cr ratio (ng/mg)	10 (−26 to 64)	—	0.6	0.9	0.6
Dichotomous outcomes					
ACR > 30 mg/g	—	2.00 (1.17 to 3.44)	0.02	0.8	0.08
Detectable A1M	—	1.13 (0.65 to 1.97)	0.8	0.7	0.9

Note: Multivariable models adjust for age, hypertension, diabetes mellitus, body mass index, hepatitis C virus infection, HIV viral load, CD4 cell count, current combination antiretroviral therapy, creatinine-based estimated glomerular filtration rate, and principal components 1, 3, and 5.

Abbreviations: A1M, α_1-microglobulin; ACR, albumin-creatinine ratio; CI, confidence interval; Cr, creatinine; HIV, human immunodeficiency virus; IL-18, interleukin 18; KIM-1, kidney injury molecule 1; NGAL, neutrophil gelatinase-associated lipocalin.

[a]Estimated percentage difference attributable to having 2 versus 0/1 *APOL1* risk alleles.

[b]Adjusted prevalence ratio among individuals with 2 versus 0/1 *APOL1* risk alleles.

[c]*P* value compares 2 versus 0/1 *APOL1* risk alleles.

[d]*P* value compares 2/1 versus 0 *APOL1* risk alleles.

[e]*P* value for trend (2 vs 1 vs 0 *APOL1* risk alleles).

genotype with longitudinal kidney outcomes. To our knowledge, this is the first study to use these urine biomarkers to distinguish associations of the *APOL1* risk genotype with glomerular and tubular injury.

The strong association of the *APOL1* risk genotype with albuminuria is consistent with results of prior investigations of individuals with mild kidney disease. Among nondiabetic African Americans in the Dallas Heart Study, individuals with 2 *APOL1* risk alleles were observed to be 2.8-fold more likely to have microalbuminuria compared with those with 0/1 risk allele.[34] The *APOL1* risk genotype has also been reported to be associated with macroalbuminuria in first-degree relatives of African Americans with nondiabetic ESRD.[35] Finally, among HIV-infected African Americans without prior AIDS, we previously found that the presence of 2 *APOL1* risk alleles was independently associated with 69% higher (95% CI, 36% to 108%) urine protein excretion compared to 0/1 risk allele.[36] The present study builds upon our prior work by localizing *APOL1*-associated kidney injury to the glomerulus rather than the tubules.

Contrary to our hypothesis, albuminuria did not appreciably attenuate the associations between the *APOL1* genotype and longitudinal kidney function decline. Because we measured albuminuria only at baseline, we cannot exclude the possibility that individuals with 2 *APOL1* risk alleles developed albuminuria prior to ascertainment of eGFR at the 4- or 8-year follow-up visits. Second, the relatively small sample size of individuals with 2 *APOL1* risk alleles may have reduced power to detect a smaller effect size of albuminuria on the longitudinal kidney outcomes. Finally, glomerular injury may be one of several pathways linking the *APOL1* genotype with the development of overt kidney disease among HIV-infected African Americans.

Although the pathophysiology underlying *APOL1*-associated kidney disease remains speculative, the presence of APOLI protein in podocytes in healthy kidney samples suggests a role for APOL1 in normal glomerular health.[10,11] APOL1 is a 43-kDa member of the Bcl-2 family of proteins, which are key regulators of programmed cell death.[37] When overexpressed in cancer cell lines, APOL1 induces autophagic cell death.[38] Recent studies suggest an important role for autophagy in the maintenance of glomerular homeostasis. Hartleben et al[39] observed that podocytes display unusually high levels of autophagic activity compared with tubular cells, and that mice lacking autophagic capability exhibit increased susceptibility to glomerular disease. Furthermore, Lan et al[40] demonstrated that overexpression of the G1 and G2 *APOL1* genetic variants in cultured human podocytes results in podocyte necrosis, and that APOL1 variant–induced podocyte injury is augmented by the presence of HIV infection. Podocyte dysregulation has been implicated in the development of HIVAN,[41] although gene-environment interactions appear to be necessary for the development of overt kidney disease. Papeta et al[42] found that the introduction of HIV to mice with genetic susceptibility to HIVAN led to alterations in the expression of specific proteins important in maintaining podocyte structure and function. Collectively, these in vitro and animal studies suggest that host *APOL1* genotype may interact synergistically with

Table 4. Associations of *APOL1* Risk Alleles With Kidney Function Outcomes in HIV-Infected African American Women

Outcome	0 Allele (n = 193)	1 Allele (n = 191)	2 Alleles (n = 47)
Baseline eGFR (mL/min/1.73 m^2)			
Mean (95% CI)	90.8 (88.0 to 93.5)	87.3 (84.4 to 90.3)	83.5 (76.1 to 91.0)
Estimated difference, 2 vs 0/1 alleles (95% CI)[a]			
Unadjusted	Reference	Reference	−5.5 (−13.0 to 1.9)
Adjusted + ACR	—	—	−4.7 (−10.4 to 1.0)
Adjusted + ACR, IL-18, KIM-1, NGAL, A1M	—	—	−4.1 (−9.4 to 1.2)
Annual change in eGFR (mL/min/1.73 m^2)			
Mean (95% CI)	−1.4 (−1.8 to −1.1)	−1.2 (−1.6 to −0.9)	−2.6 (−3.5 to −1.6)
Estimated difference, 2 vs 0/1 alleles (95% CI)[b]			
Unadjusted	Reference	Reference	−1.2 (−2.2 to −0.2)
Adjusted + ACR	—	—	−1.1 (−2.1 to −0.1)
Adjusted + ACR, IL-18, KIM-1, NGAL, A1M	—	—	−1.1 (−2.1 to −0.1)
Incident CKD			
No. of events	42	42	16
No. at risk	181	174	41
Incident rate ratio, 2 vs 0/1 alleles (95% CI)[c]			
Unadjusted	Reference	Reference	1.65 (1.08 to 2.52)
Adjusted + ACR	—	—	1.74 (1.15 to 2.64)
Adjusted + ACR, IL-18, KIM-1, NGAL, A1M	—	—	1.68 (1.15 to 2.46)
10% annual eGFR decline			
No. of events	13	11	10
No. at risk	193	191	47
Incident rate ratio, 2 vs 0/1 alleles (95% CI)[c]			
Unadjusted	Reference	Reference	3.40 (1.74 to 6.67)
Adjusted + ACR	—	—	3.20 (1.48 to 6.91)
Adjusted + ACR, IL-18, KIM-1, NGAL, A1M	—	—	2.82 (1.21 to 6.54)

Note: Adjusted models control for age, hypertension, diabetes mellitus, hepatitis C virus infection, HIV viral load, CD4 lymphocyte count, combination antiretroviral therapy use, and the kidney injury markers listed.

Abbreviations: A1M, α_1-microglobulin; ACR, albumin-creatinine ratio; CI, confidence interval; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HIV, human immunodeficiency virus; IL-18, interleukin 18; KIM-1, kidney injury molecule 1; NGAL, neutrophil gelatinase-associated lipocalin.

[a]Estimated difference in baseline eGFR attributable to having 2 versus 0/1 *APOL1* risk alleles.

[b]Estimated difference in annual eGFR change attributable to having 2 versus 0/1 *APOL1* risk alleles.

[c]Incident rate ratio for 2 versus 0/1 *APOL1* risk alleles.

HIV to modulate pathways involved in podocyte integrity.

In humans, the contributions of the *APOL1* genotype and HIV to the development and progression of glomerular disease have been studied in kidney biopsy series. In HIV-infected African Americans with non-HIVAN pathology, Fine et al[43] found that number of *APOL1* risk alleles correlated with type of non-HIVAN histopathology and risk for ESRD. However, among individuals with biopsy-confirmed HIVAN, Atta et al[44] reported no differences in renal pathology or risk for ESRD by *APOL1* genotype. The discordant findings of these studies may reflect the heterogeneity of HIV-related kidney disease in African Americans. Further studies are needed to evaluate pathophysiologic mechanisms mediating APOL1-associated kidney risk.

Of note, because APOL1 has been detected in vascular endothelium,[10] albuminuria among individuals with *APOL1* risk variants could signify the presence of subclinical vascular disease. Albuminuria is an established risk factor for cardiovascular disease, even among individuals with preserved eGFRs.[45] Recent studies evaluating associations of the *APOL1* genotype with cardiovascular outcomes have yielded conflicting results.[46] Among African Americans in the Jackson Heart Study and the Women's Health Initiative, 2 *APOL1* risk alleles were associated with a 2-fold greater risk of atherosclerotic cardiovascular disease.[47] By contrast, Langefeld et al[48] found no association between *APOL1* risk variants and prevalent cardiovascular disease in African Americans enrolled in the Systolic Blood Pressure Intervention Trial, whereas Freedman et al[49] reported lower risk for subclinical atherosclerosis and death in association with the *APOL1* risk genotype among African Americans in the Diabetes Heart Study. Understanding the interplay between *APOL1* risk genotype, HIV suppression, kidney injury, and the development of cardiovascular disease through future studies may have substantial implications for the aging HIV-infected population.

The findings of the present study do not support a strong pathogenic role for APOL1 risk variants in tubular injury and dysfunction among HIV-infected African Americans with well-preserved kidney function. However, tubular injury may play a more prominent role in the absence of HIV infection or at more advanced stages of APOL1-associated kidney disease. For instance, in the Focal Segmental Glomerulosclerosis Clinical Trial, from which persons with HIV were excluded, Kopp et al[50] found that individuals with the high-risk *APOL1* genotype had more extensive tubular atrophy and fibrosis on kidney biopsy. Furthermore, in African Americans with "hypertension-associated" nephropathy, Larsen et al[51] reported a higher prevalence of tubular atrophy and microcystic dilatation on kidney biopsy samples of individuals with 2 versus 0/1 *APOL1* risk alleles. Notably, participants in that study had advanced kidney disease, with a mean serum creatinine level of 4.3 mg/dL in individuals with 2 *APOL1* risk alleles. Additional studies are needed to validate these findings and investigate the contributions of tubulointerstitial damage to prognosis at later stages of APOL1-associated kidney disease.

Our study has several limitations. First, as a study of women, results are not generalizable to men, although there is currently no evidence to suggest a sex-based interaction between *APOL1* genotype and kidney disease risk. Second, as a cross-sectional study nested within a cohort, our study is subject to selection bias due to study dropout or competing events and we cannot make conclusions regarding causality. Third, almost half the HIV-infected African American women in the WIHS were excluded from initial analyses due to missing *APOL1* genotype or urine biomarker measurements, which could reduce power or bias results. However, our findings remained robust when we repeated the analyses using multiple imputation to include participants with missing data. Fourth, urine samples were collected more than a decade prior to biomarker measurement, so degradation over time may have contributed to the null associations between *APOL1* genotype and tubular biomarkers. Alternatively, the tubular injury markers may lack sensitivity for detection of tubular dysfunction at very early stages. However, these urine biomarker levels had strong associations with subsequent eGFR decline and mortality risk.[14,15] Finally, despite our adjusting for multiple potential confounders, the possibility of residual confounding remains.

In summary, among HIV-infected women of African ancestry, APOL1-associated kidney injury appears to localize to the glomerulus rather than tubules. Additionally, the *APOL1* risk genotype was strongly predictive of kidney function decline. Future studies should evaluate the pathophysiologic mechanisms by which variant forms of APOL1 result in glomerular injury, as well as targeted therapies to slow the progression of APOL1-mediated kidney disease.

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Contributions: Research idea and study design: VJ, RS, MGS, MME; data acquisition: MGS, MB, MHC, MN, AS, MY, PCT, CRP, MME; data analysis/interpretation: VJ, RS, CRP, RSP, MGS, MME; statistical analysis: RS; supervision or mentorship: MGS, RSP, CRP, MME. WHLK died before the manuscript was submitted; MME affirms that she contributed to data acquisition and vouches for her coauthorship status; all other authors approved the final author list. Except as noted, each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. VJ takes responsibility that this study has been reported honestly, accurately, and transparently; that no important aspects of the study have been omitted, and that any discrepancies from the study as planned have been explained.

SUPPLEMENTARY MATERIAL

Table S1: Demographic and clinical characteristics, stratified by inclusion/exclusion in *APOL1* substudy.

Table S2: Proportion of missing data for variables included in multiple imputation model.

Table S3: Adjusted associations of *APOL1* risk alleles with biomarkers using multiple imputation.

Table S4: Associations of *APOL1* risk alleles with kidney function outcomes using multiple imputation.

Table S5: Urine biomarker levels in women with eGFR $\geq$ 60, stratified by number of *APOL1* risk alleles.

Table S6: Adjusted associations of *APOL1* risk alleles with biomarkers among women with eGFR $\geq$ 60.

Figure S1: Distributions of urine KIM-1, NGAL, and A1M, stratified by number of *APOL1* risk alleles.

Item S1: Supplemental methods.

Note: The supplementary material accompanying this article (http://dx.doi.org/10.1053/j.ajkd.2015.02.329) is available at www.ajkd.org

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