APOL1 Risk Variants Predict Histopathology and Progression to ESRD in HIV-Related Kidney Disease

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ABSTRACT

With earlier institution of antiretroviral therapy, kidney diseases other than HIV-associated nephropathy (HIVAN) predominate in HIV-infected persons. Outcomes for these diseases are typically worse among those infected with HIV, but the reasons for this are not clear. Here, we examined the role of *APOL1* risk variants in predicting renal histopathology and progression to ESRD in 98 HIV-infected African Americans with non-HIVAN kidney disease on biopsy. We used survival analysis to determine time to ESRD associated with *APOL1* genotype. Among the 29 patients with two *APOL1* risk alleles, the majority (76%) had FSGS and 10% had hypertensive nephrosclerosis. In contrast, among the 54 patients with one *APOL1* risk allele, 47% had immune-complex GN as the predominant lesion and only 23% had FSGS. Among the 25 patients with no *APOL1* risk allele, 40% had immune-complex GN and 12% had FSGS. In 310 person-years of observation, 29 patients progressed to ESRD. In adjusted analyses, individuals with two *APOL1* risk alleles had a nearly three-fold higher risk for ESRD compared with those with one or zero risk alleles (P=0.03). In summary, these data demonstrate an association between *APOL1* variants and renal outcomes in non-HIVAN kidney disease, suggesting a possible use for *APOL1* genotyping to help guide the care of HIV-infected patients.

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Kidney disease is an important risk factor for morbidity and mortality among HIV-infected individuals despite highly active antiretroviral therapy (HAART).[1–3] A paucity of data, however, exists on the worldwide prevalence of CKD in this patient population. Cross-sectional studies from 31 European countries, Israel, and Argentina estimate the prevalence of CKD between 3.5% and 4.7% among HIV-infected individuals depending on the formula used to estimate GFR.[4] A recent US study using cystatin C–based estimated GFR, potentially a more sensitive method, demonstrated that much higher percentages of HIV-infected patients exhibit CKD. In this study, Choi *et al.* showed that 28% of predominantly HAART-exposed, HIV-infected patients had either impaired kidney function (defined as estimated $GFR_{CYSC} < 60$ ml/min per 1.73 m^2) or albuminuria. Importantly, kidney disease contributed to a substantial fraction (17%) of mortality in this population.[5] The finding of significant numbers of HIV-infected individuals with CKD is not particularly surprising because renal glomerular and tubular cells contain HIV mRNA and DNA, indicative of active HIV replication in kidney tissues.[6,7]

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Studies of transgenic mouse models implicate HIV infection of renal cells coupled with the appropriate host genetic background as leading to the development of HIV-associated nephropathy (HIVAN).[8,9] In humans, HIVAN occurs almost exclusively in individuals of African ancestry,[10] and hereditary factors have been implicated in its development. Freedman *et al.* reported familial clustering of ESRD in African Americans with ESRD due to HIVAN,[11] and proposed a genetic contribution to this and other forms of kidney disease in African Americans. Recently, mapping by admixture linkage disequilibrium revealed a strong statistical association between nonmuscle myosin heavy chain 9 gene (*MYH9*) polymorphisms on chromosome 22 and nondiabetic kidney disease in African Americans, particularly hypertensive nephrosclerosis, FSGS, and most notably HIVAN.[12–14] The statistical association of *MYH9* risk variants with HIVAN was particularly striking, and some researchers concluded that the single nucleotide polymorphisms (SNPs) in the S cluster of *MHY9* SNPs conferred 70%–100% of the attributable risk of HIVAN.[15] However, detailed sequencing and genotyping failed to identify specific functional mutations in *MYH9.*[16] Stronger associations were soon demonstrated between nondiabetic kidney disease in African Americans and two independent sequence variants in the nearest gene in the 3′ centromeric direction, *APOL1*.[17,18] The study by Genovese *et al.* demonstrated an odds ratio of 10.5 (95% confidence interval [95% CI], 6.0–18.4) for idiopathic FSGS and 7.3 (95% CI, 5.6–9.5) for hypertension-attributed ESRD.[18] Important additional insight came from the observation that HIVAN was absent among Ethiopians, despite the high frequencies of E and S cluster *MYH9* risk variants versus zero allele frequencies for the F risk SNP closest to the 3′ centromeric end of *MYH9* and G1 and G2 risk variants within *APOL1* itself in this population.[16,17,19] Notably, these *APOL1* risk variants were shown to encode versions of *APOL1*, which confer protection from *Trypanosoma brucei rhodesiense* infection, providing an evolutionary adaptive advantage in the face of a high burden of this pathogen.[18] Such selective advantage explains the African regional differences in *APOL1* G1 and G2 risk frequencies and also the extended pattern of linkage disequilibrium that results in association of disease risk in tagging SNPs at neighboring genomic loci.[16,18] More than 30% of African Americans likely carry the *APOL1* G1 (S342G and I384M; rs73885319 and rs60910145) or G2 (NY388-389 del; rs71785313) risk alleles.[18] Although the frequency of individuals who carry two risk alleles among healthy African Americans is approximately 12%,[17,18] 66% of those with biopsy-proven FSGS carried two risk alleles.[18] Extrapolating from the risk of *MYH9* alleles, we can predict that among untreated HIV-infected with 2 *APOL1* alleles a large proportion will develop FSGS or HIVAN.[13,20]

In a recent study of HIV-infected African Americans seen in an urban clinic, HIVAN comprised a smaller annual proportion of renal biopsies compared with the early periods of HIV infection and AIDS.[21] Similar findings have been reported from France in which the proportion of those with HIVAN has also decreased over time.[22] Therefore, with greater availability and earlier initiation of HAART, the proportion of kidney disease attributed to non-HIVAN lesions will increase. Notably, in a series of 243 kidney biopsies at the Johns Hopkins Hospital, 71% were non-HIVAN.[23]

This study focuses on HIV-infected African Americans without HIVAN to clarify associations of *APOL1* genotypes with kidney disease pathology and progression to ESRD in this population.

RESULTS

This study consisted of 98 HIV-infected African Americans diagnosed with biopsy-proven non-HIVAN kidney disease and successfully genotyped for *APOL1*. There were no significant differences in age, sex, illicit drug use, hepatitis C virus coinfection, kidney function, and proteinuria at the time of biopsy across the *APOL1* risk allele groups (Table 1). Although no difference was observed in HIV viral load or proportion of patients with suppressed viral load, the CD4+ cell count was significantly higher in those with no *APOL1* risk allele.

Pathologic Diagnoses with Two *APOL1* Risk Alleles

Twenty-nine patients (30%) were either homozygous or compound heterozygous for G1/G2. Of these, 76% had FSGS, 10% had hypertensive nephrosclerosis, and 10% had diabetic kidney disease (Tables 2 and 3). Only one patient had immune-mediated GN. Of all patients with a diagnosis of FSGS, 63% had two risk alleles.

Pathologic Diagnoses with One *APOL1* Risk Allele

Forty-four patients (45%) possessed only one *APOL1* risk allele (designated G1/0 or G2/0 in Table 2). Of these, 47% had immune-complex GN, 23% had FSGS, 9% had diabetic nephropathy, and 23% had other diagnoses (minimal change, pyelonephritis, *etc.*).

Pathologic Diagnoses with No *APOL1* Risk Alleles

Of the 25 patients with no risk allele, only 3 individuals (12%) had FSGS, whereas over 40% had immune-complex lesions. Diabetic nephropathy was diagnosed in 28% and hypertensive nephrosclerosis in 8%.

Clinical Features According to Most Common Histopathologic Diagnoses

Clinical features of FSGS, immune-complex GN, and diabetic nephropathy, the three most common diagnoses, are compared in Table 4. The three groups were similar, although those with diabetes had higher levels of proteinuria at time of biopsy and were less likely to have a history of illicit drug use (although similar hepatitis C rates). Importantly, of 22 patients with diabetes, only 14 had diabetic nephropathy. Of the remaining eight diabetic patients without diabetic nephropathy, two each

Table 1. Baseline characteristics

Characteristic	All Patients (*N*=98)	Two *APOL1* Risk Alleles (*n*=29)	One *APOL1* Risk Allele (*n*=44)	No Risk Alleles (*n*=25)	*P* Value
Mean age, yr (SD)	47.0 (8.3)	46.3 (8.4)	46.2 (8.8)	48.4 (7.3)	0.24
Women	32 (33)	5 (17)	19 (43)	8 (32)	0.07
Diabetes	22 (22)	5(17)	7 (16)	10(40)	0.05
Hypertension	61 (62)	20 (69)	24 (55)	17 (68)	0.37
Hepatitis C	54 (55)	13 (45)	19 (66)	12 (48)	0.15
Hepatitis B	7 (7)	4 (14)	2(5)	1(4)	0.25
Illicit drug use history	50 (52) [*n*=96]	12 (41)	27 (63) [*n*=43]	11 (46) [*n*=24]	0.16
ACEi/ARB use at biopsy	25 (25)	8 (29)	8 (18)	9 (36)	0.22
Mean HIV viral load, 1000 copies/ml (SD)	123 (231)	87 (173) [*n*=27]	145 (247) [*n*=43]	123(264) [*n*=23]	0.26
HIV viral load <50 copies/ml	29 (31) [*n*=93]	7 (26) [*n*=27]	19 (44) [*n*=43]	6 (26) [*n*=23]	0.18
Mean CD4+ cell count, cells/mm^3 (SD)	305 (257)	262 (156)	269 (257)	429 (322)	0.03
Mean prebiopsy nadir CD4+ cell count, cells/mm^3 (SD)	183 (217)	170 (151)	169 (178)	229 (336)	0.75
Mean proteinuria, mg/24 h or mg/g creatinine (SD)	2399 (2718)	1771 (1612)	2774 (3215)	2460 (2744)	0.59
Mean serum creatinine, mg/dl (SD)	2.7 (1.8)	2.5 (0.9)	3.0 (2.4)	2.4 (1.3)	0.76
Mean eGFR (CKD-EPI), ml/min per 1.73 m^2 (SD)	45.4 (31.7)	40.9 (18.4)	47.9 (40.3)	45.0 (24.0)	0.92

Data are *n* (%) unless otherwise specified. CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated GFR.

Table 2. Biopsy findings by *APOL1* genotype

	G1/G1 (*n*=7)	G2/G2 (*n*=3)	G1/G2 (*n*=19)	G1 Hetero (*n*=27)	G2 Hetero (*n*=17)	No Risk allele (*n*=25)
FSGS	5 (71)	3 (100)	14 (74)	7 (27)	3 (18)[a]	3 (12)
HTN nephrosclerosis	1 (14)		2 (11)			4 (8)[b]
Diabetic nephropathy	1 (14)[c]		2 (11)[c]	4 (15)[c]		7 (28)[d]
Immune-complex GN			1 (5)	11 (42)	9 (53)	10 (40)
lupus-like				−3	−2	−2
postinfectious				−6[e]	−1	−1
IgA			−1	−2	−2	−3
MPGN					−1	−1
membranous					−2	−1
nonspecific					−1	−2
Other				5 (19)	5 (29)	1 (4)

Data are *n* (%) unless otherwise specified. MPGN, membranoproliferative GN.
[a]All patients with concurrent findings (one resolving postinfectious GN, one with HTN disease, and one with evidence of remote immune-complex GN).
[b]Two patients with other diagnoses (tenofovir toxicity, acute tubular necrosis) that are likely the primary diagnoses.
[c]All patients with concurrent FSGS lesions
[d]One patient with FSGS in the context of very advanced diabetic nephropathy.
[e]Two IgA-dominant patients.

(as shown in Table 4) had FSGS and immune-complex GN. Two of the other four patients (not shown in Table 4) had hypertensive nephrosclerosis, one had interstitial nephritis, and one acute tubular necrosis.

Risk of ESRD Associated with *APOL1*

During 310 person-years of follow-up, 29 participants progressed to ESRD. Individuals with two *APOL1* risk alleles experienced faster progression to ESRD (Figure 1). In unadjusted analyses, individuals with two *APOL*1 risk alleles had a nearly two-fold greater risk of ESRD compared with those with one or zero or risk alleles (Table 5); however, this did not reach statistical significance (P=0.08). Conversely, lower kidney function at the time of kidney biopsy was associated with significantly greater risk of ESRD (hazard ratio [HR], 10.47 per $\log_{10}$-1 ml/min per 1.73 m^2 lower; P<0.001). There was a trend for higher risk of ESRD among individuals with proteinuria at the time of kidney biopsy; however, this did not reach statistical significance (HR, 5.94; P=0.08). In adjusted analyses, individuals with two *APOL1* risk alleles had a nearly three-fold greater risk of ESRD compared with those with one or zero risk alleles (P=0.03). Baseline kidney function remained significantly associated with ESRD risk (HR, 10.48 per $\log_{10}$-1 ml/min per 1.73 m^2 lower; P=0.009). Exposure to renin angiotensin-aldosterone system blockers at the time of biopsy or after biopsy did not alter this association. Suppression of viral load after the time of biopsy also had no effect on this outcome.

Table 3. Biopsy findings by number of *APOL1* risk alleles

Lesion	Two Risk Alleles (*n*=29)	Two versus Zero *P* Value	One Risk Allele (*n*=44)	No Risk Alleles (*n*=25)	One versus Zero *P* Value
FSGS	22 (76)	<0.001	10 (23)	3 (12)	0.28
Hypertension nephrosclerosis	3 (10)	0.54	0	4 (8)	<0.01
Diabetic nephropathy	3 (10)	0.1	4 (9)	7 (28)	0.04
Immune-complex GN	1 (3)	0.001	20 (47)	10 (40)	0.66
Other	0	0.28	10 (23)	1 (4)	0.04

Data are *n* (%) unless otherwise specified.

Table 4. Patient characteristics associated with most common biopsy findings

Characteristic	FSGS (*n*=35)	Immune-Complex GN (*n*=31)	Diabetic Nephropathy (*n*=14)	*P* Value
No. of *APOL1* risk alleles				<0.001
0 allele	3 (8)	10 (32)	7 (50)	
1 allele	10 (29)	20 (64)	4 (29)	
2 alleles	22 (63)	1 (3)	3 (21)	
Mean age, yr (SD)	47.3 (7.2)	45.5 (9.4)	46.8 (9.1)	0.45
Women	9 (26)	12 (39)	6 (43)	0.39
Diabetes	2 (6)	2 (6)	14 (100)	<0.001
Hypertension	26 (74)	16 (52)	11 (78)	0.08
Hepatitis C	16 (46)	20 (64)	7 (50)	0.30
Hepatitis B	1 (3)	2 (6)	1 (7)	0.74
Illicit drug use history	17 (49)	19 (63)	2 (15)	0.01
ACEi/ARB use at biopsy	8 (23)	7 (24)	6 (43)	0.33
ACEi/ARB use after biopsy	20 (64)	12 (44)	8 (67)	0.23
Mean HIV viral load, 1000 copies/ml (SD)	68 (151)	149 (217)	26 (44)	0.02
HIV viral load <50 copies/ml at biopsy	14 (40)	7 (23)	2 (18)	0.22
HIV viral suppression after biopsy	[*n*=32]	[*n*=28]	[*n*=11]	0.61
undetectable >90% of time	16 (50)	10 (36)	3 (27)	
intermittent suppression	6 (19)	5 (18)	3 (27)	
Mean CD4+cell count, cells/mm^3 (SD)	312 (239)	299 (224)	402 (362)	0.66
Mean prebiopsy nadir CD4+cell count, cells/mm^3 (SD)	179 (190) [*n*=30]	180 (133) [*n*=29]	269 (442) [*n*=11]	0.77
Mean proteinuria, mg/24 h or mg/g creatinine (SD)	1920 (1686)	2284 (2648)	5173 (3663)	0.005
Mean serum creatinine, mg/dl (SD)	2.5 (1.3)	2.9 (2.5)	2.6 (1.4)	0.99
Mean eGFR (CKD-EPI), ml/min per 1.73 m^2 (SD)	41.5 (22.1)	49.9 (43.2)	42.8 (28.5)	0.93

Data are *n* (%) unless otherwise specified. ACEi, ACE-inhibitor; ARB, angiotensin receptor blocker; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated GFR.

DISCUSSION

In this study, the *APOL1* risk variants were useful in predicting renal histopathology in HIV-infected African Americans without HIVAN. The association of *APOL1* risk alleles with nondiabetic ESRD, including HIVAN, has been previously described[17]; previous studies, however, have not examined those with non-HIVAN kidney disease, a growing proportion of HIV-infected individuals with kidney disease. We chose to focus on HIV-infected patients for several reasons. First, the changing histopathology in these patients renders predicting underlying renal disease on the basis of currently available noninvasive clinical parameters quite difficult. Second, few clinical centers routinely perform kidney biopsies in HIV-infected persons and therefore genotyping may help bridge the information gap between invasive and noninvasive clinical parameters, with potential future management implications. Finally, this patient population, in particular, has the potential to obtain the largest benefit upon genotyping for these *APOL1* variants due to the availability of antiretroviral drugs.

This study suggests that the absence of risk alleles strongly predicts lesions other than FSGS. Of those undergoing biopsy with two risk alleles, 76% had FSGS, whereas this diagnosis was far less frequent in those with one or no risk allele. Interestingly, immune-complex GNs were commonly seen in those with one or no risk allele but seen in only one patient with two risk alleles.

The reason for the preponderance of immune-complex GN in those without two risk alleles is not clear. One could hypothesize that the absence of *APOL1* risk alleles may somehow modify the immune status in a way that enhances

immune-complex GN in the context of HIV and other infections. This would most likely require invoking a relationship between innate immunity and immune-complex disease. Of interest, there is a case report of a patient in whom trypanosomal disease due to *Trypanosoma brucei evansi* (normally not a human pathogen) was attributed to a null state of the gene.[24] No further details were provided regarding kidney or other autoimmune or immune-injury disease. It is more likely, however, that those with two risk alleles develop FSGS and manifest with kidney disease early that is diagnosed and preempts immune-complex GN. We note that many of the patients with FSGS in all risk groups had other well established risk factors for kidney injury, including poorly controlled hypertension, episodes of AKI, illicit drug use with cocaine and heroin, and exposure to nephrotoxins with possible acute interstitial nephritis, which was seen concurrently on some biopsies.

We also note that diabetic nephropathy (the third most common diagnosis in this sample set) occurred in only 14 of 22 patients with diabetes. Although, as expected, the presence of diabetes makes a diagnosis of diabetic nephropathy more likely, one cannot exclude other diagnoses on the basis of the presence of diabetes. As in non-HIV populations, nondiabetic renal disease is not uncommon in patients with diabetes,[25] although patients with diabetes who undergo kidney biopsies tend to have clinical features that suggest a diagnosis other than diabetic nephropathy. These features such as absence of retinopathy, presence of hematuria or other laboratory results suggesting nondiabetic kidney disease were not studied in this cohort. However, patients with diabetic nephropathy did have higher levels of proteinuria at the time of biopsy (more than double that of the other groups) that may have prompted biopsy in search of a diagnosis other than diabetes. Ultimately, the presence of diabetes should be considered as just one of the many factors that may predict renal histopathology. Additional studies are needed to further elucidate its role as predictor in this clinical setting, especially in view of the emerging increase in diabetes in the HIV-infected population.[26]

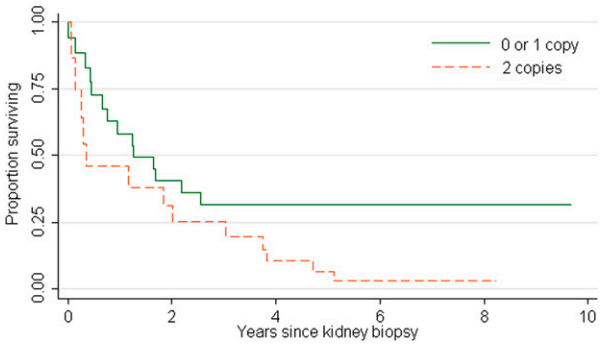

Figure 1. Age-adjusted renal survival by number of *APOL1* risk alleles.

The biologic mechanism of kidney disease risk associated with the *APOL1* risk alleles remains to be determined.[16] Genetic analysis studies to date strongly favor a recessive mode, wherein two risk alleles are required for the phenotype of significant risk association.[18] Prior reports of a possible association under dominant or additive modes of inheritance did not take into account the compound heterozygous state of G1/G2 risk.[17] Therefore, the results of this study are also best understood in the context of a recessive mode of inheritance, wherein no significant differences in histopathologic diagnoses were noted comparing the one versus or zero risk allele states, as shown in Table 3. Nevertheless, this formulation does not preclude epistatic interactions with other genetic loci, or gene-by-environment interactions, which may result in kidney disease risk with a single *APOL1* risk allele. We also note that even a purely recessive mode of inheritance for disease risk association does not inform as to whether the biologic mechanism of kidney disease risk is a "loss of function" or "gain of injury" mechanism, and information concerning activities of the protein product of *APOL1*, apoL1, are potentially consistent with either. ApoL1 is homologous to the Bcl2 family of proteins and is involved in apoptosis and autophagy.[27–29] MRNA encoding apoL1 has been demonstrated in liver, lung, placenta, and endothelial cells, as well

Table 5. Hazard ratios of ESRD associated with *APOL1* risk allele

Variable	Univariate		Multivariate	
	Hazard Ratio (95% CI)	*P* Value	Hazard Ratio (95% CI)	*P* Value
APOL1, 2 versus 0/1 risk alleles	1.98 (0.93–4.24)	0.08	2.86 (1.12–7.32)	0.03
Age, per year increase	0.97 (0.29–1.02)	0.29	0.98 (0.93–1.04)	0.50
Female	0.79 (0.33–1.85)	0.59	—	—
Hepatitis C antibody seropositivity	1.02 (0.49–2.12)	0.96	1.14 (0.44–2.91)	0.79
Diabetic	1.38 (0.47–4.02)	0.56	0.79 (0.17–3.60)	0.76
Hypertensive	0.78 (0.37–1.63)	0.50	0.66 (0.27–1.65)	0.37
HIV-1 RNA level, per $\log_{10}$ 1 copy/ml higher	1.28 (0.94–1.74)	0.36	1.21 (0.76–1.94)	0.42
CD4+cell count, per 1 cell/mm^3 higher	1.00 (0.99–1.00)	0.10	1.00 (0.99–1.00)	0.92
Baseline eGFR, per $\log_{10}$-1 ml/min per 1.73 m^2 lower	10.47 (2.87–38.15)	<0.001	10.48 (1.79–61.27)	0.009
Proteinuric at baseline	5.94 (0.80–43.94)	0.08	3.63 (0.46–28.54)	0.22

Proteinuria defined as spot urine protein/creatinine ratio >200 mg/g. eGFR, estimated GFR.

as weakly in the kidney, heart, pancreas, and macrophages.[30] The presence in macrophages may suggest that the protein plays a role in immune responses. ApoL1 is a secreted lipoprotein and circulates on HDL3 complexes.[28,30–32] Various mechanisms have been proposed whereby *APOL1* mutations may cause glomerulosclerosis.[33] Mutated apoL1 particles may bind less tightly to the circulating HDL3, undergo glomerular filtration and proximal tubular resorption, and thereby cause kidney disease, or may bind differently to other circulating proteinuric factors.[34] Alternatively, endogenous apoL1 in the renal epithelium-like cell may cause apoptosis or cause autophagic cell death.[35]

Patients with two and one risk alleles had significantly lower CD4+ cell counts than patients with zero risk alleles. The lower level of CD4+ cells increases the probability of infectious episodes, which, in turn, cause the elaboration of the following cytokines: IFNα, IFNβ, and IFNγ, as well as TNFα.[35] All four molecules are recognized potent stimulators of APOL1 gene upregulation.[30,36–38]

Patients with two *APOL1* risk variants were more likely to develop ESRD. Not surprisingly, baseline kidney function was the strongest predictor of progression to ESRD. The association of two risk alleles with ESRD is likely related to the type of disease (*i.e.,* FSGS) more commonly seen with these risk alleles, although one cannot rule out an effect of having the risk alleles on progression itself. Because of the low numbers of FSGS in the low-risk allele groups (one or zero risk alleles), we were unable to look at progression focused among those with FSGS in a separate analysis. Although the progression may be related more to the disease type than to an effect of the specific isoform of apoL1, this finding is important because presence of the high-risk alleles seems to not only predict disease type but also disease outcome, as was also observed in non-HIV-infected cohorts.[39]

There were several limitations to this study. First, it should be noted that the study sample is limited to individuals who had undergone kidney biopsy, and this may not be representative of all HIV-infected patients with kidney disease. Patients who do not have a clinical indication or who have a contraindication to kidney biopsy were not included, and their histopathologic diagnoses may differ. Therefore, we cannot exclude the possibility that the distribution of histopathologic diagnoses might be different in a sample set based on a prospective collection of all patients consenting to kidney biopsy. Nevertheless, the results provide a rigorously derived picture of the state of the relationship between the *APOL1* genotype and non-HIVAN kidney disease histopathology in practice that reflects actual clinical implementation of kidney biopsy. Second, we did not have a comparable non-HIV-infected group to make comparisons regarding diagnoses. However, the diagnoses seen in this study were quite characteristic of diagnoses seen in the general African-American population, particularly FSGS, which is the most common cause of nephrotic-range proteinuria in African Americans.[40–42] Third, this was a retrospective analysis with the inherent limitations of such analyses, one of which is the potential for selection bias noted above. Fourth, the role of *APOL1* in the poorer outcomes seen in those with two risk alleles is difficult to determine because the diseases and thus prognoses in this group differed from the other risk groups. In addition, the clinical indications for biopsy may have differed, although baseline kidney function was similar across risk groups, and the urine protein excretion was actually lower in those with two risk alleles.

This study shows the potential clinical implications for *APOL1* genotyping in this population and needs to be further defined. A patient with no risk allele seems unlikely to develop FSGS and is more likely to present with other disorders such as immune-complex GN when kidney disease is present. *APOL1* genotyping could eventually inform timing of HAART initiation, decisions regarding kidney biopsy, use of renin angiotensin-aldosterone system inhibitors, and other interventions relevant to the management of those with existing kidney disease. Clinical trials are needed to address these issues, which represent the next phase of translating these findings to the possible benefit of patient care and well being.

CONCISE METHODS

Study Design and Population

Percutaneous native kidney biopsies from African-American HIV-infected adults performed at the Johns Hopkins Hospital between January 1996 and December 2009 were assessed. In patients with repeated biopsies, only the first was considered for this study. A total of 271 such biopsies were available; only those with a biopsy report noting non-HIVAN lesions were included in this study (N=140). The pathologic criteria for designation as HIVAN were glomerular segmental or global collapse in at least one glomerulus and podocyte hypertrophy/hyperplasia with or without microcystic tubular dilatation, as previously described.[43] The presence of segmental glomerular sclerosis in the absence of any glomerular collapse or microcystic tubular dilation defined FSGS. Immunofluorescence and electron microscopy were reviewed in each case. No tissue was obtained from 17 biopsies, and genotyping was unsuccessful in an additional 23. Demographic, clinical, and laboratory data were abstracted from the electronic patient record. This study was approved by the Institutional Review Board of the Johns Hopkins University School of Medicine and Rambam Medical Center.

Histopathologic Review of Biopsy Material

Histopathology of all genotyped cases was reviewed by a pathologist (M.K.) who had no knowledge of the previously reported histopathologic diagnosis. An additional 20 HIVAN cases were added to slides that this pathologist reviewed to reduce the bias that may have existed by knowing that the study was of non-HIVAN participants. After pathologic review, findings were compared with the original pathology reports (all performed by pathologists other than M.K.). If findings differed from the original report, a second pathologist (L.C.R.) reviewed the slides. All of the added nonstudy HIVAN cases were again designated as such. Six non-HIVAN biopsy results were

reclassified to other non-HIVAN diagnoses during the pathologic review. Two patients originally diagnosed as non-HIVAN were determined to have HIVAN during pathologic review for this study and were excluded.

Genotyping Assay for Formalin-Fixed Paraffin-Embedded Tissue

DNA Extraction from Formalin-Fixed Paraffin-Embedded Tissue

Tissue sections of 10-μm thickness were obtained from formalin-fixed paraffin-embedded tissues. Genomic DNA was extracted using the QuickExtract FFPE DNA Extraction Kit (Epicentre Biotechnologies) according to the manufacturer's protocol. DNA was concentrated by DNA Clean & Concentrator 5 (Zymo Research) according to the manufacturer's protocol and kept at 4°C before use.

High-Resolution Melting Assays

High-resolution melting assay and PCR methods, including PCR primers used, are detailed in the Supplemental Material.

Statistical Analyses

Statistical analyses were performed using Stata 9.2 statistical software (StataCorp, College Station, TX). Baseline characteristics were compared according to the number of *APOL1* risk alleles using the Kruskal–Wallis test for continuous variables and the chi-squared test for categorical variables. Variables with skewed distributions were $\log_{10}$-transformed.We evaluated the association of *APOL1* risk alleles with time to ESRD using survival analysis methods. Survival times were determined from the time of kidney biopsy to ESRD (defined as an estimated GFR $<$15 ml/min per 1.73 m^2 or initiation of dialysis). Participants were censored at the last available visit, death, or the date of April 20, 2011. Kaplan–Meier estimates were calculated, and incident ESRD rates were compared between those with two *APOL1* risk alleles versus those with one or no risk alleles. Unadjusted and adjusted Cox proportional hazards models were constructed to determine the HR for ESRD and corresponding 95% CI. Covariates in the final model were selected on the basis of their clinical and statistical significance on univariate analyses. The proportionality assumption was tested by log-log plots and plots of Schoenfeld residuals versus time.

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DISCLOSURES

None.

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