APOL1 Risk Variants Are Strongly Associated with HIV-Associated Nephropathy in Black South Africans

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ABSTRACT

APOL1 variants are associated with HIV-associated nephropathy and FSGS in African Americans. The prevalence of these variants in African populations with CKD in HIV-1 infection has not been investigated. We determined the role of *APOL1* variants in 120 patients with HIV-associated nephropathy and CKD and 108 controls from a South-African black population. Patients with CKD were selected on the basis of histology. Genotypes were successfully determined for *APOL1* G1 and G2 variants and 42 single nucleotide polymorphisms, including 18 ancestry informative markers, for 116 patients with CKD (96.7%; 38 patients with HIV-associated nephropathy, 39 patients with HIV-positive CKD, and 39 patients with HIV-negative CKD), and 108 controls (100%). Overall, 79% of patients with HIV-associated nephropathy and 2% of population controls carried two risk alleles. In a recessive model, individuals carrying any combination of two *APOL1* risk alleles had 89-fold higher odds (95% confidence interval, 18 to 912; $P<0.001$) of developing HIV-associated nephropathy compared with HIV-positive controls. Population allele frequencies were 7.3% for G1 and 11.1% for G2. *APOL1* risk alleles were not significantly associated with other forms of CKD. These results indicate HIV-positive, antiretroviral therapy–naïve South-African blacks with two *APOL1* risk alleles are at very high risk for developing HIV-associated nephropathy. Further studies are required to determine the effect of *APOL1* risk variants on kidney diseases in other regions of sub-Saharan Africa.

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CKD is an important public health problem worldwide. African Americans experience high rates of CKD arising from diabetic nephropathy, hypertension-attributed CKD, FSGS, and HIV-associated nephropathy (HIVAN).[1–3] HIV-positive individuals of African descent have an 18-fold to 50-fold increased risk of developing CKD[4] and an 18-fold increased risk of developing HIVAN, suggesting that genetic factors play an important role in susceptibility for HIVAN.[2]

HIVAN is considered to be a morphologic variant of FSGS characterized by segmental and global

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glomerular collapse, hypertrophy and hyperplasia of visceral glomerular epithelial cells, and severe tubulointerstitial disease.[5] In the absence of effective antiretroviral therapy (ART), approximately 50% of patients with HIVAN progress to ESRD within 2 years.[6,7] The introduction of ART has led to a substantial decline in the incidence of HIVAN in the United States.[6,7] In sub-Saharan Africa, HIVAN is the most common cause of kidney disease morbidity and mortality in HIV-positive ART-naïve patients.[8,9]

Mapping by admixture linkage disequilibrium studies for FSGS, HIVAN, and nondiabetic ESRD among African Americans led to the identification of a genomic region on chromosome 22q12 with very strong association with HIVAN, FSGS, and other forms of nondiabetic and hypertension-attributed ESRD.[10,11] Further studies identified three codon-changing variants in the *APOL1* gene, encoding ApoL1, with extremely strong associations with HIVAN (odds ratio [OR], 29; 95% CI, 13.1 to 68.5), FSGS (OR, 17; 95% CI, 11 to 26.5), and ESRD (OR, 7; 95% CI, 6 to 10) for homozygotes or compound heterozygotes carrying two risk alleles in Americans with African ancestry.[12–14] *APOL1* renal risk variants have been shown to increase the rate of progression to ESRD in persons with CKD, including FSGS, diabetic CKD, and hypertension-attributed CKD.[13,15]

The *APOL1* risk alleles comprise two tightly linked missense variants G1 (rs73885319; p.S342G and rs60910145; p.I384M) and G2, a 6-bp in-frame deletion removing two amino acids (rs71785313; p.N388del:Y389del) in the last exon of the *APOL1* gene.[12] The G1 and G2 alleles range in frequency from 0% to approximately 45% for G1 and to approximately 20% for G2 in different geographical regions of sub-Saharan Africa with the lowest frequencies in East Africa and the highest frequencies observed in West Africa.[13,16–19] The prevailing hypothesis is that the G1, and to a lesser extent G2, alleles have been under recent selection in West Africa by trypanosomes or other extracellular pathogens.[12] This study aimed to determine the prevalence of *APOL1* risk variants and the effect of these variants on HIVAN and CKD in black South Africans in a setting of high HIV-1 prevalence.

RESULTS

This study included 228 adult individuals of black African ancestry from Johannesburg, South Africa. Characteristics of the CKD patient groups, all of whom were verified by histology, and HIV-positive controls are presented in Table 1. HIVAN was distinguished from HIV-positive FSGS by the presence of glomerular capillary collapse and glomerular visceral epithelial cell proliferation affecting at least one glomerulus, together with microcystic tubular dilation and interstitial inflammation. Median eGFR was significantly lower in patients with HIVAN (11.9 ml/min per 1.73 m^2) compared with all other patients with CKD (33.5 ml/min per 1.73 m^2 and 56.6 ml/min per 1.73 m^2 for HIV-positive and HIV-negative patients, respectively) and HIV-positive controls (P=0.001). Measures of kidney function were not available for the population control group; they were all apparently healthy individuals who gave consent to be enrolled for population genetic studies as control participants. The CKD patient groups and HIV-positive control group did not differ significantly by age (34.6, 36.1, 36.4, and 38.8 years, respectively) and sex at biopsy or study enrollment ($P>0.05$) (Table 1). All HIV-positive patients with CKD were ART naïve; however, 75% of the HIV-positive control group had been initiated on ART before recruitment into the study. Median viral load (log10 copies/ml) levels were highest in patients with HIVAN (5.1 log copies/ml) compared with HIV-positive patients with CKD (4.7 log copies/ml) and undetectable median levels in HIV-positive controls. Median CD4 counts were lowest in patients with HIVAN (92 cells/mm^3) compared with HIV-positive patients with CKD (244.5 cells/mm^3) and HIV-positive controls (371.0 cells/mm^3) (Table 1). *APOL1* genotypes were determined for 96.7% of patients with CKD (HIVAN, n=38; HIV-positive CKD, n=39; and HIV-negative CKD, n=39) and 100% of controls (HIV-positive controls, n=54; and population controls, n=54) (Table 2). Genotype distributions did not differ significantly from Hardy–Weinberg equilibrium expectations in HIV-positive and population control groups ($P>0.05$). Single nucleotide polymorphism (SNP) and haplotype frequencies are shown in Figure 1. Two infrequent haplotypes were observed for G1; these were confirmed by Sanger sequencing and by a repeat of the TaqMan assay. Because of the close proximity of the two G1 SNPs, recombination between them is a rare event. The haplotype G-G-I ($G1^{GM}$), comprising the derived allele at both rs73885319 (p.S342G) and rs60910145 (p.I384M) and the ancestral insertion (I) allele at rs71785313, was the most frequent G1 configuration (6.4% in the population controls and 52.6% in the HIVAN group). A novel haplotype, $G1^{+M}$ (A-G-I), confirmed by Sanger sequencing of the PCR product, was observed as a compound heterozygote ($G1^{+M}/G1^{GM}$) in a single HIV-positive patient with FSGS. Although Kopp *et al.*[13] showed that the effect size of the $G1^{GM}$ and $G1^{G+}$ haplotypes are equivalent, the renal risk of $G1^{+M}$ cannot be determined; therefore, we excluded this individual from further analysis in this study.

In the population control group, the allele frequencies were 7.3% for the G1 risk allele for rs73885319 and 11.1% for the G2 deletion allele (Figure 1). The combined frequency of the G1 and G2 risk alleles was highly enriched in patients with HIVAN (90.6%) compared with HIV-positive controls (20.8%) ($P=8.0\times10^{-20}$) and the population control group (18.4%) ($P=8.0\times10^{-22}$) (Figure 1). Similar distortions were observed for carriage of two *APOL1* risk alleles; 78.9% of the patients with HIVAN were homozygous (G1/G1 or G2/G2) or compound heterozygotes (G1/G2) compared with 3.7% of the HIV-positive controls ($P=1.2\times10^{-14}$) and 1.9% of the population controls ($P=8.9\times10^{-16}$) (Table 2). The distribution of G0, G1, and G2 genotypes and risk alleles among patients with CKD and controls are shown in Table 2. The distribution of genotypes was not statistically different when comparing the HIV-positive patients with CKD with HIV-positive controls or

Table 1. Characteristics of study participants and controls

Characteristic	HIVAN (n=39)[d]	HIV-Positive CKD (n=40)[e]	HIV-Positive Controls (n=54)[f]	P Value[g]	HIV-Negative CKD (n=41)[e]	Population Controls (n=54)[h]
Sex, *n* (%)						
Men	16.0 (41.0)	18.0 (42.9)	15.0 (28.0)	0.07	24.0 (58.1)	24.0 (44.4)
Women	23.0 (59.0)	22.0 (57.1)	39.0 (72.0)	0.73	17.0 (41.9)	30.0 (55.6)
Mean age (SD)	34.6 (8.3)	36.1 (9.3)	38.8 (7.1)	0.62	36.4 (11.0)	38.5 (8.6)
Viral load (log copies/ml)[a]	5.1 (4.5–5.3)	4.7 (3.8–4.9)	Undetectable	0.001	—	—
CD4 (cells/mm^3)[b]	92.0 (76–195)	244.5 (71.5–351.5)	371.0 (215.0–545.0)	0.001	—	—
eGFR (ml/min per 1.73 m^2)[c]	11.9 (7.5–22.6)	33.5 (15.9–68.8)	121.6 (103.3–146.9)	0.001	56.6 (8.3–101.7)	Not available
Serum creatinine (μmol/L)	534.0 (262.0–799.0)	233.0 (106.0–388.0)	66.0 (55.0–77.0)	0.001	155.0 (91.0–778.0)	Not available

Data are given as the median (interquartile range) unless otherwise indicated.
[a]Patients with HIVAN (n=30), HIV-positive patients with CKD (n=27), and HIV-positive controls (n=45).
[b]Patients with HIVAN (n=26), HIV-positive patients with CKD (n=32), and HIV-positive controls (n=41).
[c]eGFR was calculated according to the modified Modification of Diet in Renal Disease formula.
[d]HIV-associated nephropathy (collapsing FSGS).
[e]See Table 3 for histologic diagnosis for HIV-positive and HIV-negative CKD.
[f]HIV-positive controls with no kidney disease.
[g]Kruskal–Wallis test comparing HIV-positive patients with HIV-positive controls.
[h]Population controls were from the Division of Human Genetics, National Health Laboratory Service, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand.

Table 2. *APOL1* genotype distribution among patients with HIVAN, HIV-positive patients with CKD, HIV-negative patients with CKD, and controls

Genotype	HIV-Positive Patients and Controls			HIV-Negative Patients and Controls	
	HIVAN[a]	CKD[b]	HIV-Positive Controls	CKD[c]	Population Controls
0 risk alleles					
G0/G0	2 (5.3)	22 (56.4)	34 (63.0)	25 (64.1)	36 (66.7)
1 risk allele	6 (15.8)	11 (28.2)	18 (33.3)	13 (33.3)	17 (31.5)
G0/G1	5 (13.2)	4 (10.3)	4 (7.4)	6 (15.4)	7 (13.0)
G0/G2	1 (2.6)	7 (17.9)	14 (25.9)	7 (17.9)	10 (18.5)
2 risk alleles	30 (78.9)	6 (15.4)	2 (3.7)	1 (2.6)	1 (1.9)
G1/G1	8 (21.0)	2 (5.1)	0 (0.0)	0 (0.0)	0 (0.0)
G1/G2	19 (50.0)	2 (5.1)	0 (0.0)	1 (2.6)	0 (0.0)
G2/G2	3 (7.9)	2 (5.1)	2 (3.7)	0 (0.0)	1 (1.9)
Total	38	39	54	39	54

Data are given as *n* (%). The distribution of *APOL1* genotypes and fractions with one or two risk alleles for the study groups is shown.
[a]One patient did not pass genotyping.
[b]The single HIV-positive patient with CKD carrying the G1^{+M} (A-G-I) haplotype is excluded from this table and one patient did not pass genotyping.
[c]Two patients did not pass genotyping.

comparing HIV-negative patients with CKD with population controls (Table 2).

We explored the distribution of *APOL1* G1 and G2 alleles among the histologically diagnosed HIV-positive and HIV-negative patients with CKD (Table 3, Supplemental Table 1). For the HIV-positive group, there was no significant association between *APOL1* genotypes and HIV-positive patients with FSGS (OR, 2.13; 95% CI, 0.03 to 44.30; P=0.48) and HIV-associated immune complex kidney disease (HIVICK) (OR=5.60; 95% CI, 0.4 to 86; P=0.13) compared with HIV-positive controls. There was also no significant association for primary FSGS in the HIV-negative group (OR, 6.30; 95% CI, 0.04 to 248.70; P=0.26) compared with population controls (Tables 3 and 4). To increase statistical power, we combined HIV-positive patients with FSGS (n=13) and primary FSGS (n=9) and compared this with all controls (n=108) and still did not observe any significant association with *APOL1* G1 and G2 alleles in a recessive model (P=0.20) (Table 4). Because the point ORs for *APOL1* association with primary FSGS and HIVAN differed in this study from those reported by Kopp *et al.*,[13] we used a Woolf test for homogeneity to determine whether the ORs were significantly different. Although the ORs in this study were higher for HIVAN and lower for FSGS compared with the ratios observed in African Americans, the differences were not statistically significant (P=0.56 and P=0.21 for FSGS and HIVAN, respectively). We also compared ORs for HIV-positive FSGS seen in this study with data from Fine *et al.*[20] for African Americans with HIV-positive FSGS compared with a population control group from the study by Kopp *et al.*[13] (OR, 24.5; 95% confidence interval [95% CI], 9.7 to 65.1); P=4.3×10^{-14}); the Woolf test gave a P value of 0.07.

We assessed the effect size and statistical significance for carriage of one or two *APOL1* risk variants by comparing the distribution of two versus one or zero copies of *APOL1* G1 and G2 risk alleles in patients and controls in a recessive model (Table 4). Consistent with previous studies, the association of

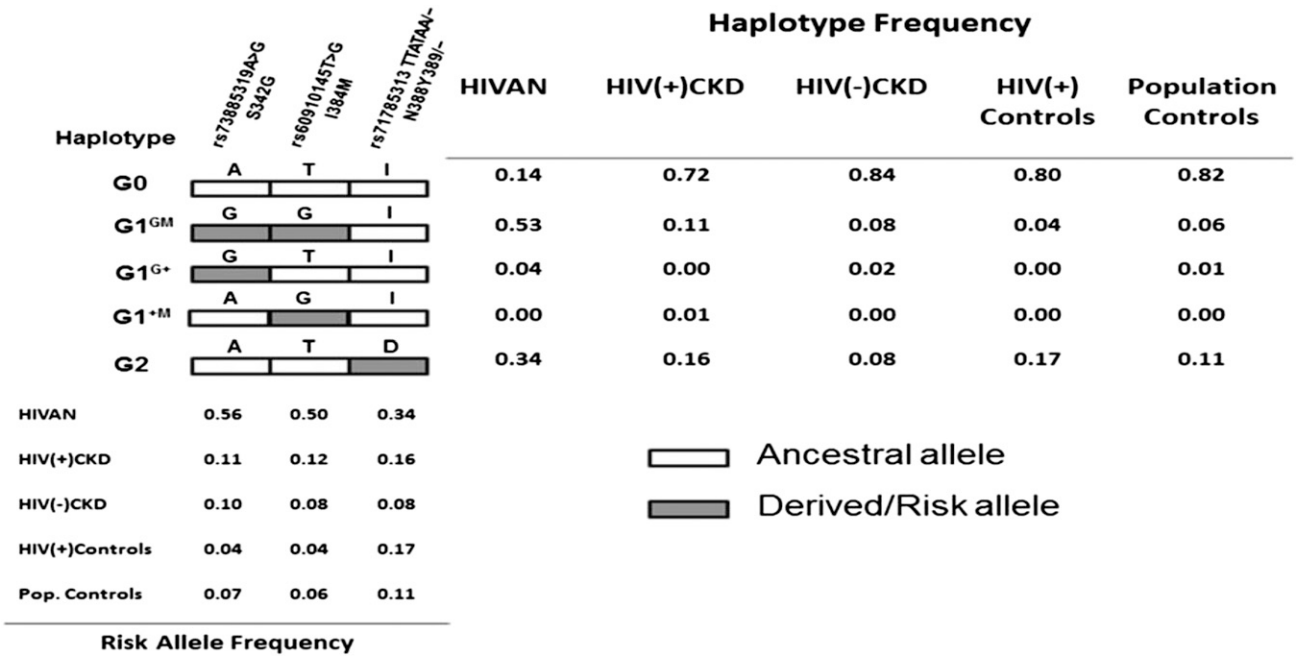

Figure 1. Distribution of *APOL1* haplotypes and risk alleles. Five *APOL1* haplotypes are observed. The ancestral haplotype, denoted as G0, has three ancestral alleles. The $G1^{GM}$ haplotype has two missense alleles. The $G1^{G+}$ haplotype has one missense risk allele at rs73885319. The $G1^{+M}$ has one missense variant at rs60910145. The G2 haplotype has the 6-bp deletion risk allele at rs71785313. The risk allele and haplotype frequencies are shown for patients with HIVAN, HIV-positive patients with CKD and HIV-negative patients with CKD, and HIV-positive controls and population controls (PCs).

APOL1 genotypes with HIVAN best fits a recessive genetic model. Carriage of two copies of *APOL1* renal risk alleles was a strong predictor of HIVAN (OR, 89.1; 95% CI, 17.68 to 911.72; $P=1.24\times10^{-14}$). The predictor was so strong that carriage of even one renal risk allele increased the risk of HIVAN with marginal statistical support (OR, 5.49; 95% CI, 0.87 to 61.14; $P=0.05$) (Table 4). The results for HIVAN remained highly significant after correcting for age, sex, and genetic ancestry (Table 4).

Examination of the independent effect sizes (ORs) of the G1 and G2 alleles (Figure 2) indicated that the effect size of

Table 3. Association between *APOL1* risk alleles and various glomerular diseases

Glomerular Disease	HIV-Positive Patients and Controls					HIV-Negative Patients and Controls				
	No. of *APOL1* Risk Alleles			OR (95% CI)	*P* Value	No. of *APOL1* Risk Alleles			OR (95% CI)	*P* Value
	0	1	2	2 versus 1 or 0 risk alleles		0	1	2	2 versus 1 or 0 risk alleles	
Controls (*n*=108)	34 (63)	18 (33)	2 (4)	—	—	36 (67)	17 (32)	1 (2)	—	—
HIVAN (*n*=38)	2 (5)	6 (16)	30 (79)	89 (17.7 to 912)	1.2×10^{-14}	—	—	—	—	—
Other CKD (*n*=78)	22 (57)	11 (28)	6 (15)	3.8 (0.6 to 42)	0.13	25 (64)	13 (33)	1(3)	1.4 (0.02 to 11)	>0.99
FSGS (*n*=22)	9 (69)	3 (23)	1 (8)	2.1 (0.03 to 44)	0.48	5 (56)	3 (33)	1 (11)	6 0.3 (0.08 to 527)	0.26
HIVICK (*n*=12)	4 (33)	5 (42)	3 (25)	5.6 (0.4 to 86)	0.13	—	—	—	—	—
Other GN (*n*=27)[a]	7 (70)	3 (30)	0 (0)	0.0 (0 to 30)	>0.99	10 (59)	7 (41)	0 (0)	0 (0 to 124)	>0.99
Other kidney diseases (*n*=17)[b]	2 (50)	0 (0)	2 (50)	21 (0.2 to 2029)	0.11	10 (77)	3 (23)	0 (0)	0 (0 to 210)	>0.99

Data are given as *n* (%) unless otherwise indicated. The *APOL1* genotype frequencies and associations, tested with the Fisher exact test, are shown for various glomerular diseases among HIV-positive and HIV-negative patients and general population controls. The only glomerular disease that showed a significant association with *APOL1* risk alleles was HIVAN. Of note, HIV-positive FSGS, HIV-negative FSGS, and HIVICK were not significantly associated with *APOL1* risk alleles, although group sizes were small. Analyses were adjusted for age, sex, and ancestry. *P* values indicate probability by the Fisher exact test.

[a]Other GN is as follows: HIV-positive: membranoproliferative GN (*n*=1), membranoproliferative GN consistent with C3 glomerulopathy (*n*=1), and membranous GN (*n*=8); and HIV-negative: IgA nephropathy (*n*=1), lupus nephritis (*n*=3), membranoproliferative GN (*n*=2), and membranous GN (*n*=11).

[b]Other kidney diseases are as follows: HIV-positive: benign nephrosclerosis (*n*=1), global glomerulosclerosis (*n*=1), minimal change disease (*n*=1), and thrombotic microangiopathy (*n*=1); and HIV-negative: benign nephrosclerosis (*n*=1), oxalosis (*n*=1), global glomerulosclerosis (*n*=2), minimal change disease (*n*=5), nodular glomerulosclerosis (*n*=1), severe arterial nephrosclerosis with secondary FSGS (*n*=1), and thrombotic microangiopathy (*n*=2).

Table 4. *APOL1* risk allele associations with HIVAN, HIV-associated FSGS, and HIV-negative FSGS (primary FSGS)

No. of Risk Alleles	HIV-Positive CKD Patient Groups (*n*=51) versus HIV-Positive Controls (*n*=54)						Primary FSGS (*n*=9) versus Population Controls (*n*=54)		All FSGS (*n*=22) versus All Controls (*n*=108)	
	HIVAN (*n*=38)			HIV-Positive FSGS (*n*=13)			Primary FSGS (*n*=9)		HIV-Positive FSGS (*n*=13) and Primary FSGS (*n*=9)	
	OR (95% CI)	*P* Value	Adjusted *P* Value[a]	OR (95% CI)	*P* Value		OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
1 versus 0	5.49 (0.87 to 61.14)	0.05	0.13	0.63 (0.10 to 2.98)	0.74		1.3 (0.18 to 7.42)	>0.99	0.86 (0.25 to 2.64)	>0.99
2 versus 0	200.87 (27.62 to 3119.17)	1.5×10^{-14}	1.9×10^{-3}	1.85 (0.03 to 39.57)	0.53		6.70 (0.08 to 577.00)	0.26	3.27 (0.25 to 31.46)	0.22
2 versus 1	40.50 (7.09 to 446.94)	8.7×10^{-08}	1.8×10^{-4}	2.82 (0.04 to 73.20)	0.44		5.0 (0.05 to 467.90)	0.34	3.74 (0.26 to 40.67)	0.20
2 versus 1 or 0 recessive model	89.1 (17.68 to 911.72)	1.2×10^{-14}	1.5×10^{-5}	2.13 (0.03 to 44.30)	0.48		6.30 (0.04 to 248.70)	0.26	3.45 (0.27 to 32.23)	0.20

ORs (95% CIs) are shown for the association between *APOL1* risk alleles and particular glomerular diseases. *P* values are from Fisher's exact test unless otherwise noted.
[a]Analyses were adjusted for age, sex, and ancestry.

G2 was weaker than G1; however, it is worth noting that G1/G2 compound heterozygotes are equivalent in effect size to G1/G1.

Population stratification analyses using a principal components analysis (PCA) showed that the distribution of patients with HIVAN and HIV-positive control groups was not significantly different along the top-two eigen axes ($P>0.05$), confirming that the high association observed is not due to an undetected population substructure that might cause false positive associations (Supplemental Figure 1).

DISCUSSION

This is the first study that correlates genotypic information with phenotypes in HIV-associated CKD in Africa, a region with the highest prevalence of HIV-1 infection.[21] The study shows an extremely strong association between *APOL1* variants, found only on African chromosomes, and biopsy-confirmed HIVAN, an aggressive form of kidney disease that rapidly progresses to ESRD if untreated. Among the patients with HIVAN, 79% carried two copies of *APOL1* risk alleles compared with 2% in the general population. Notably, the effect size of *APOL1* risk alleles for HIVAN in black South Africans (OR, 89.1; 95% CI, 17.8 to 911.72; $P=1.24\times10^{-14}$) was numerically higher than in African Americans (OR, 29.2; 95% CI, 13.1 to 68.5; $P=6.0\times10^{-22}$), although the CIs overlapped.[13] To our knowledge, this is the strongest effect size ever reported for common variants with complex diseases.[22]

Although there is no statistical difference between effect sizes in South Africans with HIVAN compared with African Americans with HIVAN,[13] both the frequency of *APOL1* risk alleles (79% versus 72%) and ORs (89 versus 29) are elevated in South Africa. Although these differences may reflect statistical fluctuation, *APOL1* may also have a stronger gene–environment interaction in this setting, which warrants further investigation. First, this strong effect might be related to HIV-1 subtype C. HIV-1 subtype C is the dominant strain in Southern and Eastern Africa and South Asia, whereas HIV-1 subtype A is common in West Africa and subtype B is dominant in Europe, Australia, and the Americas.[23] HIV-1 subtype C is highly virulent and accounts for approximately 50% of all HIV infections worldwide and 98% of HIV infections in South Africa, with corresponding higher viral loads.[23,24] High HIV RNA levels are correlated with a decline in CD4 T cells and with the development of HIVAN in individuals of African ancestry.[25] Second, in resource-limited settings, late initiation of ART may predispose at-risk individuals to HIVAN; several studies have shown that effective rollout of ART can reduce the occurrence of HIVAN.[6,7,9] Furthermore, all of the HIV-positive patients with CKD in this study were ART naïve. Third, there are regional differences in nutrition, environmental exposures, and other viral infections that may modify the effects of *APOL1* variants on the kidney. Fourth, there might be other genetic variants unique to or more common in this population that interacts with *APOL1*-driven susceptibility to HIVAN.

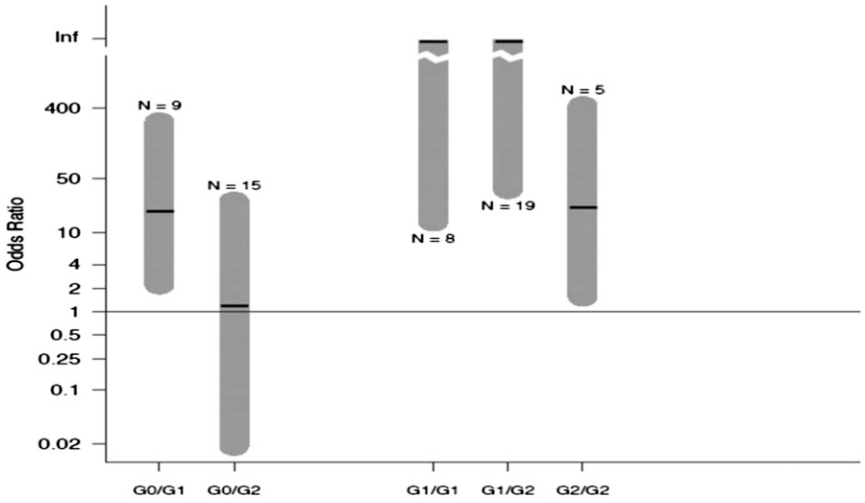

Figure 2. ORs for the effect sizes for HIVAN with different combination of *APOL1* G1 and G2 alleles. ORs with CIs for association of different strata of G1–G2 genotypes with HIVAN patients compared with participants carrying no G1 or G2 risk alleles (+/+). For G1/G1 and G1/G2, the OR is infinity, because these genotypes were only observed in patients.

This study also showed a weak effect with one *APOL1* risk allele in HIVAN (OR, 5.49; 95% CI, 0.87 to 61.14; P=0.05), consistent with previous studies.[12,13,26] An effect from one risk allele would be consistent with a gain of injury and/or toxicity of these variants in kidney cells that manifests with a strong environmental insult, such as HIV infection, as opposed to a purely recessive effect that might suggest a loss of function.

There was little or no significant *APOL1* association with multiple histologic phenotypes of HIV-positive patients with CKD and HIV-negative patients with CKD. Previous studies in African Americans have reported ORs for primary FSGS with two *APOL1* risk alleles as 10.5 (95% CI, 6.0 to 18.1) and 17 (95% CI, 11 to 26.5; $P=1.3\times10^{-48}$).[12,13] In addition, other studies also demonstrated higher association of *APOL1* risk alleles with FSGS.[20,27] It is possible that HIV infection is an incidental finding to primary FSGS in black South Africans, with FSGS being the commonest histology reported in children and adults worldwide.[28,29] However, others have suggested that FSGS may be part of the spectrum of histology in response to HIV infection.[30] It is also possible that due to the high HIV prevalence in South Africa, susceptible HIV-infected individuals who are ART naïve may develop HIVAN and not FSGS. In addition, we found that *APOL1* risk allele status was not associated with HIVICK, a result in concordance with that of a study in African Americans that observed two *APOL1* risk alleles in only 1 of 31 patients with HIVICK.[31]

HIV is a powerful driver of *APOL1* nephropathy, as seen by the large proportion of individuals with HIVAN who carry two risk allele genotypes and the strong effect sizes observed. Furthermore, the frequencies were similar for two risk allele genotypes in HIVAN (79% in this study and 72% in Kopp *et al.*[13] study), although the two risk allele genotype frequencies are markedly lower in the South-African (2%–4%) compared with the African-American general population (12%–14%).[13] It was previously estimated that 50% of HIV-positive patients with two *APOL1* risk alleles who do not receive ART will develop HIVAN in their lifetime, suggesting the magnitude of the interaction.[13] Nevertheless, it is worth noting that HIV-positive patients with two risk alleles do not necessarily develop HIVAN and that approximately 20% of patients with HIVAN carry one or no *APOL1* risk allele, suggesting that there are more factors to be considered. Clearly, HIV infection interacts strongly with *APOL1* variants, although the molecular mechanism is largely unknown, and this interaction is similar in South Africa and the United States.

The *APOL1* G1 and G2 alleles show distinct distributions among various African and African-derived populations. With a robust population sample comprising 216 chromosomes (including HIV-positive controls), we were able to establish that the G1 allele (rs73885319) frequency is approximately 7.3%, much lower than reported in West Africa (Yoruba and Igbo from Nigeria), in whom the frequencies are approximately 45% and 30%, respectively, or in African Americans where the G1 frequency is approximately 20%.[13,17] This may be as a result of relaxation of selection pressures exerted by trypanosomiasis or other pathogens in regions inhabited by ancestors of this South-African population or may represent introgression of the variant alleles into southern African populations as a result of gene flow from the Bantu expansion.[32,33] In this study, G1 and G2 alleles have a combined allele frequency of 18.4%, indicating that in the general black population, 32% of individuals carry one risk allele and 2% carry two risk alleles. The risk of developing HIVAN is substantial for individuals living with HIV infection and possessing two *APOL1* risk alleles.[13,27] We also detected a rare, recombinant haplotype, $G1^{+M}$ (A-G-I), in the heterozygous state ($G1^{GM}/G1^{+M}$), in a single individual with HIV-positive CKD, which has not been previously reported in the 1000 Genomes Project or in previous studies of African populations, summarized by Thomson *et al.*[33] and Limou *et al.*[34]

The molecular mechanisms by which *APOL1* variants cause kidney disease remain unclear. This protein is expressed in podocytes and proximal tubular epithelial cells in normal kidneys and arteriolar endothelial cells and in vascular smooth muscle cells in HIVAN and FSGS biopsies.[35] The G1 and G2 variant proteins, respectively, have altered and deleted amino acids in the *APOL1*-binding region of the trypanosomal serum resistance–associated (SRA) protein.[12] By so doing, the trypanosomal SRA is not able to bind *APOL1,* and hence is exposed to the trypanolytic activity of ApoL1. This suggests that the risk variants

in the SRA domain of *APOL1* act by a gain of injury or a toxicity mechanism on podocytes or vascular cells.

A study by Nichols *et al.*[36] showed that despite the recessive mode of inheritance observed in *APOL1* kidney disease, there is evidence suggesting that *APOL1* risk variants act as toxic gain-of-function mutations and that HIV triggers the immune system by activating exogenous IFNs that in turn increase ApoL1 expression in cells, leading to the development of kidney disease.[36] Having carried out our study in a high HIV prevalence setting, with predominantly HIV-1 subtype C, puts HIV as an important environmental factor inducing *APOL1*-mediated kidney disease, supporting a two-hit model for *APOL1*-associated disease expression.[37] This study highlights the need to consider gene–environment interaction in the interpretation of genome-wide association studies for other common diseases.

Our study also highlights the need for HIV screening, surveillance, and implementation of World Health Organization (WHO) recommendations for earlier ART initiation to reduce the burden of HIVAN and other forms of HIV-related kidney disease in Africa. At present, guidelines for the treatment of HIV infection in South Africa suggest that ART be instituted for individuals with WHO clinical stage 3 and 4 disease and HIV-specific manifestations (*e.g.,* HIV-related malignancies, kidney or other organ involvement, and hepatitis B and C infection) and in all HIV-positive individuals with CD4 counts <350 cells/μl.[38]

There are several limitations to our study. We lacked sufficient numbers of primary or HIV-positive patients with FSGS to detect the effects of the G1 and G2 risk alleles (a sample size of 95 patients with FSGS and 95 controls would be required for 80% power to detect an OR of 5, α 0.05). Our control group comprised healthy population controls who were assumed to have normal kidney function; however, kidney function was not assessed. Although it is theoretically possible that undetected renal disease could reduce power, random controls are commonly used in genetic studies and it is unlikely that healthy individuals in this group had undetected glomerulopathy particularly given the low frequency of individuals carrying two *APOL1* renal risk alleles in our control group.

In conclusion, this study shows an extremely strong association between *APOL1* variants and biopsy-confirmed HIVAN driven by interaction between *APOL1* and untreated HIV infection, the first such study in Southern Africa.

CONCISE METHODS

Study Participants

Archived kidney biopsies obtained from patients aged >18 years were retrieved from the Division of Anatomic Pathology at the University of the Witwatersrand, according to their histologic diagnosis and HIV status (Table 1). Renal histologic diagnoses were made on the basis of standard diagnostic criteria. Histology was confirmed by an independent pathologist (A.B.F.). HIVAN was defined by the presence of glomerular capillary collapse and glomerular visceral epithelial cell proliferation affecting at least one glomerulus, together with microcystic tubular dilation and interstitial inflammation. HIVICK was defined by the presence of glomerular endocapillary cell proliferation together with an increased deposition of at least one Ig, thus including HIV-positive IgA nephropathy. Other histopathologic groups included the following: HIV-positive and HIV-negative FSGS, other GN (which included membranoproliferative GN and membranoproliferative GN consistent with C3 glomerulopathy, membranous GN, HIV-negative IgA nephropathy, and lupus nephritis,) and other kidney disease (which included thrombotic microangiopathy, nodular glomerulosclerosis, minimal change disease, arterionephrosclerosis and benign nephrosclerosis, oxalosis, and global glomerulosclerosis).

Blood samples were collected prospectively from 54 volunteers of black ethnicity and of similar age and sex to the patients with CKD for each of the two control groups: a control group (HIV-positive controls) comprising HIV-positive individuals with no clinical evidence of CKD (normal renal function; no proteinuria) from Charlotte Maxeke Johannesburg Academic Hospital and a population control group from the National Health Laboratory Service who were all apparently healthy individuals who gave consent to be enrolled for population genetic studies as control participants. All HIV-positive individuals with CKD were ART naïve and 75% of the HIV-positive control group was receiving ART. Exclusion criteria included patients aged <18 years, patients with a history of diabetes, and persons who were nonblack South Africans. This study was approved by the University of the Witwatersrand Human Research Ethics Committee (medical; approval numbers M111185 and M10745). All prospective participants (controls) provided signed informed consent.

Genomic DNA Extraction from Formalin-Fixed Paraffin-Embedded Kidney Tissue and Peripheral Blood Samples

The All Prep QIAamp DNA formalin-fixed paraffin-embedded tissue kit (Qiagen, Chatsworth, CA) was used to purify genomic DNA from formalin-fixed paraffin-embedded kidney samples, and a modified in-house salting-out procedure[39] was used to extract genomic DNA from peripheral blood samples.

SNP Genotyping

The *APOL1* SNPs G1 (rs73885319 and rs60910145) and the G2 indel (rs71785313) were genotyped using the TaqMan SNP genotyping system (Applied Biosystems, Foster City, CA), whereas 42 SNPs (18 ancestry informative markers and 24 other SNPs from chromosome 22 not in linkage disequilibrium with the risk variants) were genotyped using the Illumina BeadXpress SNP system (Illumina Inc., San Diego, CA). Sanger sequencing was used to confirm unusual haplotype configurations. Each SNP was tested for deviation from Hardy–Weinberg equilibrium using a chi-squared goodness-of-fit test in the population control group.

Population Stratification

Population stratification was assessed based on 42 SNPs distributed throughout the genome including chromosome 22 using the PCA module of Eigensoft software.[40] For these analyses, chromosome 22

SNPs were limited to $r^2<0.05$ with each other. SNPs defining the *APOL1* risk genotypes were excluded from the population stratification analyses.

Terminology

For *APOL1* haplotypes, we used the terms $G1^{G+}$ (carrying the derived risk allele at S342G only), $G1^{GM}$ (containing the derived risk alleles at S342G and I384M), and $G1^{+M}$ (containing only the derived allele at I384M). It has not been determined whether I384M confers risk in the absence of S342G.[13] For *APOL1* alleles, we use the terms G0 for the ancestral, nonrisk (+/+) allele, G1 for the risk allele of S342G that occurs on two G1 haplotypes ($G1^{G+}$ and $G1^{GM}$), and G2 for the deletion allele (Figure 1).

Statistical Analyses

The effect of *APOL1* risk genotypes on kidney disease was tested by using the Fisher's exact test, and by logistic regression adjusting for age, sex, and the first two eigen values obtained from a PCA of genetic ancestry. Informed by previous association results, we primarily tested recessive models (comparing individuals with two risk alleles, *i.e.*, G1/G1, G1/G2, or G2/G2, against all others), but also tested other models (dominant; and one versus zero, two versus zero, two versus one, and two versus one or zero risk alleles). Comparisons were made between HIV-positive patients and HIV-positive controls and between HIV-negative patients and population controls, comparing the distribution of two risk alleles (explanatory exposure) to zero or two risk alleles (no exposure). The Woolf test was used to test for homogeneity of the ORs in South-African and African-American FSGS populations.

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DISCLOSURES

None.

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