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APOL1 Renal Risk Variants and Kidney Function in HIV-1–Infected People From Sub-Saharan Africa

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Introduction: *APOL1* G1 and G2 alleles have been associated with kidney-related outcomes in people living with HIV (PLHIV) of Black African origin. No APOL1-related kidney risk data have yet been reported in PLHIV in West Africa, where high *APOL1* allele frequencies have been observed.

Methods: We collected clinical data from PLHIV followed in Burkina Faso ($N = 413$) and in the ANRS-12169/2LADY trial (Cameroon, Senegal, Burkina Faso, $N = 369$). *APOL1* G1 and G2 risk variants were genotyped using TaqMan assays, and *APOL1* high-risk (HR) genotype was defined by the carriage of 2 risk alleles.

Results: In West Africa (Burkina Faso and Senegal), the G1 and G2 allele frequencies were 13.3% and 10.7%, respectively. In Cameroon (Central Africa), G1 and G2 frequencies were 8.7% and 8.9%, respectively. *APOL1* HR prevalence was 4.9% in West Africa and 3.4% in Cameroon. We found no direct association between *APOL1* HR and estimated glomerular filtration rate (eGFR) change over time. Nevertheless, among the 2LADY cohort participants, those with both *APOL1* HR and high baseline viral load had a faster eGFR progression ($\beta = -3.9[-7.7$ to $-0.1]$ ml/min per 1.73 m^2 per year, $P < 0.05$) than those with low-risk (LR) genotype and low viral load.

Conclusion: Overall, the *APOL1* risk allele frequencies in PLHIV were higher in the West African countries than in Cameroon, but much lower than previously reported in some Nigeria ethnic groups, which strongly advocates for further investigation in the African continent. This study suggested that the virological status could modulate the *APOL1* impact on kidney function, hence reinforcing the need for early therapeutic interventions.

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The United Nations Program on HIV/AIDS estimated the number of PLHIV in 2019 at 38 (32.7–44.0) million worldwide, with Africa carrying two-thirds (25.7 million) of this burden, mainly in sub-Saharan Africa.[1] Antiretroviral treatments (ARTs) have significantly reduced HIV-related morbidity and mortality; however, with increased life expectancy among PLHIV, noninfectious co-morbidities, such as

cardiovascular, metabolic, and kidney diseases, have also increased. Chronic kidney disease (CKD) prevalence in PLHIV was estimated at 6.4% worldwide and 14.6% in West Africa.[2] CKD occurrence increases by 2-fold the risk of death among PLHIV, and people of Black African ancestry are particularly affected.[3,4] In the United States, African American PLHIV were 10 to 18 times more likely to develop HIV-associated nephropathy than their European counterparts.[5,6] Two *APOL1* coding alleles, termed G1 and G2 and found only on African-ancestry haplotypes, have been associated with a spectrum of CKD from HIV-associated nephropathy to focal segmental glomerular sclerosis, nondiabetic end-stage kidney disease, hypertension-attributed nephropathy, and increased proteinuria.[7–12] Notably, *APOL1* HR genotypes are strongly associated with HIV-associated nephropathy in untreated PLHIV of African descent with odds ratios ranging from 29 to 89 in the United States and in South Africa, respectively.[10–12] Moreover, a study in African American PLHIV revealed that patients with the HR genotypes experienced faster kidney function declines, with greater decline among those with a lack of sustained virological suppression.[13]

This study aimed to provide, for the first time, data on the distribution of *APOL1* risk variants and their impact on kidney function among treated PLHIV in different settings from West and Central Africa, as high frequencies of these variants were previously reported in West Africa.[14,15]

METHODS

We performed an observational cohort study. Data were collected from the following 2 cohorts: (i) a hospital cohort of PLHIV in Burkina Faso (day care unit [DCU] of Bobo-Dioulasso) and (ii) a cohort of HIV+ patients enrolled in a clinical trial (ANRS-12169/2LADY trial).

DCU Cohort

The first cohort is the DCU of the *Centre Hospitalier Universitaire Sourô Sanou* in Bobo-Dioulasso, Burkina Faso. The DCU, created in 2005, is part of the Infectious Disease Department and specialized in PLHIV care. The *Ensemble pour une Solidarité Thérapeutique Hospitalière en Réseau* hospital partnership initiative has supported the implementation of an electronic medical database used to monitor PLHIV care in DCU from 2007. Routine clinical follow-up visits were done every 6 months, and all clinical (general condition, symptoms, diagnosis, height, weight, blood pressure, body temperature), biological (blood count, CD4 count, glycemia, creatinine, cholesterol, triglycerides), and therapeutic (ART, cotrimoxazole prophylaxis, other treatments) data were recorded in real time by the physicians.

In this study, we included adult patients (≥18 years old) infected with HIV-1, followed in the cohort for at least 2 years, with at least 3 plasma measurements of creatinine (Figure 1a). Participants without creatinine measurement at baseline were excluded from the analyses. Patients were naive to any ART treatment at their first visit at the DCU between January 1, 2007, and December 31, 2016. For ART initiation, the 3 most prescribed treatment regimens were as follows: zidovudine (AZT) + lamivudine (3TC) + efavirenz (EFV) or nevirapine (NVP) (40.0%); tenofovir disoproxil fumarate (TDF) + emtricitabine (FTC) or lamivudine (TDF + FTC or TDF + 3TC) + EFV/NVP (31.9%); and stavudine (d4T) + 3TC + EFV/NVP (15.4%).[16] Eligible patients who came for a routine consultation between January 1, 2018, and December 31, 2018, were invited to participate in the study. After informed written consent, a blood specimen was collected for the genetic analyses ($N = 413$).

ANRS-12169/2LADY Cohort

The second cohort is from the ANRS-12169/2LADY trial,[17] which aimed to evaluate the efficacy and safety of 3 ART combinations in PLHIV who failed a first-line treatment in Africa (Cameroon, Senegal, and Burkina Faso). The participants were adults (≥18 years old) failing first-line ART that did not contain TDF or a protease inhibitor. Their creatinine clearance (Cockcroft-Gault equation) was ≥50 ml/min at baseline. The 2LADY trial follow-up visits were scheduled at weeks 4, 12, 24, 36, and 48, and every 6 months thereafter, until the end of the study. Visits included clinical (general condition, height, weight, blood pressure, body temperature) and biological (blood count, creatinine, CD4 count, glycemia, cholesterol, triglycerides, proteinuria, phosphoremia, HIV viral load) evaluation. A total of 451 patients were included and randomized to receive either TDF + FTC + darunavir-ritonavir (DRVr), TDF + FTC + lopinavir-ritonavir (LPVr) or abacavir (ABC) + didanosine (ddI) + LPVr between January 2010 and September 2012 and were followed in a period of almost 5 years.[18] Blood samples from patients were stored at −80 °C in the laboratory of the UMI 233 TransVIHMI laboratory in Montpellier, France. All consenting participants with a stored buffy coat blood sample and at least 3 creatinine measurements during the follow-up were included in this study ($N = 369$; Figure 1b).

Genetic Analyses

All genetic analyses were performed at the Molecular Genetics Epidemiology laboratory in Frederick, Maryland. DNA was extracted from blood samples

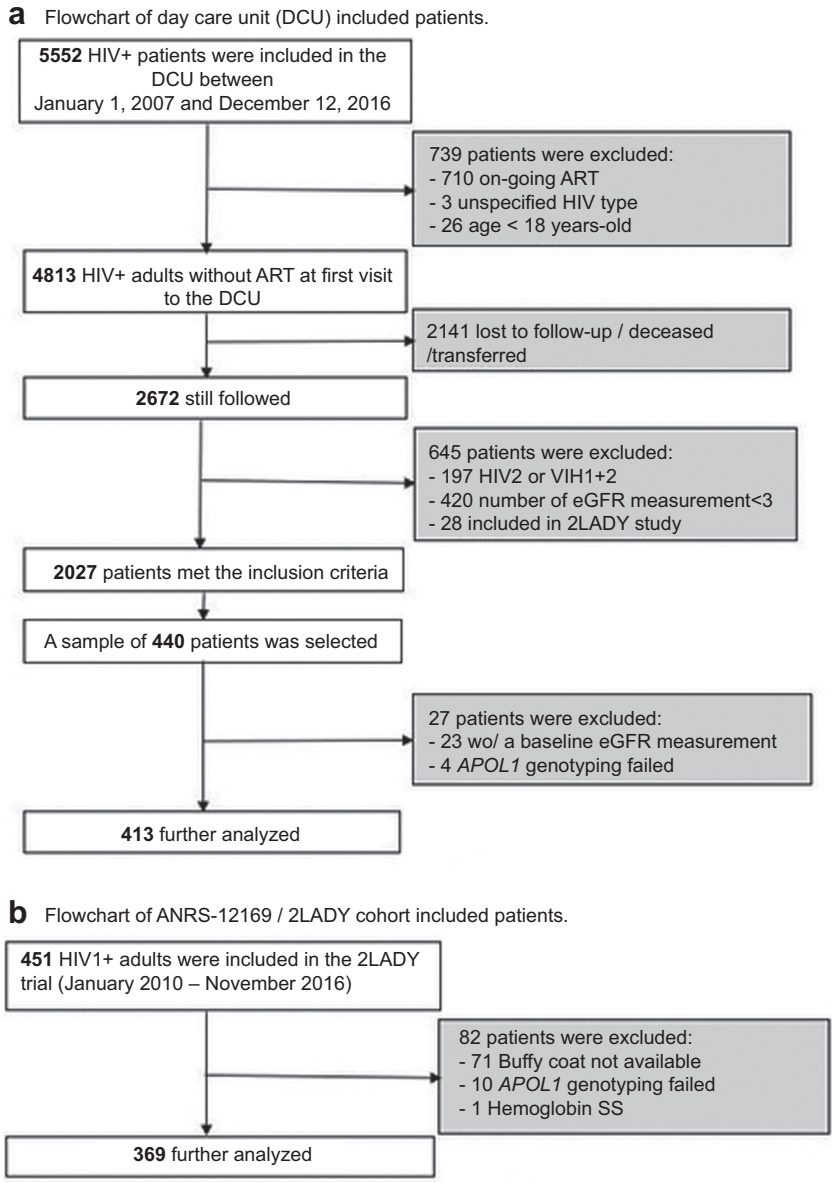

Figure 1. Study design for the (a) DCU and (b) 2LADY cohort study groups. The DCU patients were HIV+ adults initiating ART for the first time at baseline, who were subsequently followed for a median of 6.1 years—the subgroup of interest ($n = 440$) was notably selected for availability of kidney function-related data. The 2LADY patients were HIV+ adults whose first-line ART failed and who were enrolled in a second-line ART trial—they were monitored for a median of 4.8 years. All patients with available DNA were enrolled in this study. ART, Antiretroviral treatment; DCU, day care unit; eGFR, estimated glomerular filtration rate.

and purified using the Qiagen Plasmid Midi kit. The genotyping of *APOL1* G1 (rs73885319, p.S342G, and rs60910145, p.I384M) and G2 (rs71785313, p.N388Y389/–) was performed with TaqMan. The HR genotype is defined by the carriage of 2 risk alleles (G1/G1, G2/G2, or G1/G2) and the LR genotype by the carriage of 0 or 1 risk allele (G0/G0, G0/G1, or G0/G2).[14]

Estimation of GFR

The most widely used methods to measure serum creatinine are colorimetric and enzymatic methods on automated analyzer and isotope dilution-mass spectrometry. Creatinine methods based on automated colorimetric or enzymatic methods are generally used in clinical laboratories. The compensated Jaffe (colorometric) method was developed to minimize nonspecific interferences,[19,20] but the enzymatic method reveals better analytical performance, better specificity with less interference, and better reproducibility.[19] In this study, creatinine was assayed locally using isotope dilution-mass spectrometry for the 2LADY clinical trial participants and using the compensated Jaffe-based method implemented in routine medical follow-up for the DCU cohort participants.

Table 1. Distribution of participants according to the number of carried *APOL1* renal risk alleles

	Number of *APOL1* risk alleles carried				
	0 (G0/G0)	1 (G0/G1 + G0/G2)		2 (=HR) (G1/G1 + G2/G2 + G1/G2)	
Study site	*n*	*n*	*P* [CI_{95}] (%)	*n*	*P* [CI_{95}] (%)
Day care unit cohort	233	158	38.3 [33.7–43.1]	22	5.3 [3.5–8.0]
2LADY cohort					
All sites	245	112	30.4 [25.9–35.3]	12	3.3 [1.9–5.6]
Burkina Faso	29	17	36.2 [23.5–51.1]	1	2.1 [0.3–14.3]
Senegal	16	12	41.4 [24.6–60.4]	1	3.4 [0.2–22.3]
Cameroon	200	83	28.3 [23.4–33.8]	10	3.4 [1.8–6.2]
Burkina Faso[a]	262	175	38.0 [33.7–42.6]	23	5.0 [3.3–7.4]
West Africa[b]	278	187	38.2 [34.0–42.6]	24	4.9 [3.3–7.2]

HR, high risk; P, prevalence.
[a]Burkina Faso = Burkina Faso from the 2LADY and day care unit cohorts combined;
[b]West Africa = Burkina Faso + Senegal; Cameroon is representing Central Africa in our study.
The carriage of 0 or 1 risk allele defines the low-risk genotypes, when the carriage of 2 risk alleles defines the HR genotype. The detailed frequency for each genotype is provided in Supplementary Table 4B.

According to the currently accepted standards, the eGFR was estimated using the CKD-EPI equation[21] without the correction for race[22] as recently revealed as the best estimate of kidney function in Black Africans.[23–26] CKD was defined as the persistence of eGFR <60 ml/min per 1.73 m^2 in a 3-month period, corresponding to the G3a to G5 stage definition of the Kidney Disease Quality Outcome Initiative classification.[27,28]

Statistical Analyses

Baseline patient characteristics were compared by *APOL1* risk status (HR vs. LR) using the Mann-Whitney test for continuous variables and the χ^2 or the exact Fisher tests for categorical variables. These tests were also used to compare the West Africa and Cameroon cohorts.

We evaluated the association of the *APOL1* genotype with eGFR at baseline using linear regression models and with annual eGFR change during follow-up using linear mixed regression models. Mixed models included random intercept and slope to account for correlation between repeated measurements of kidney function (eGFR). We constructed multivariable regressions (i.e., adjusted analyses) to account for potential confounding risk factors for development of kidney disease. These variables included age, hypertension, glycemia, CD4 count, and HIV viral load. eGFR at baseline was also included in the models evaluating eGFR changes.

We evaluated effect modification between *APOL1* risk status and the immuno-virological status of the participants by evaluating interactions between *APOL1* genotype and CD4 count at baseline, categorized as < or ≥200 cells/μl and between *APOL1* and viral load at baseline categorized as < or ≥5 log/ml.

All statistical analyses were performed using the Stata software (version 15, Stata Corp., College Station, TX).

For power analysis, we used the R package "longpower."[29] Considering the *APOL1* HR prevalences >20% reported in Nigeria, we assumed a prevalence of 20% for HR genotype in our study population.[30] Therefore, a minimum of 350 patients (70 for HR group and 280 for LR group) was required to have a slope difference of 1 ml/min per 1.73 m^2 per year in changes in eGFR between HR and LR groups, with an α risk = 0.05 and power ≥80%.

RESULTS

Participant Characteristics

A total of 413 participants from the DCU cohort (Burkina Faso) and 369 patients from the 2LADY cohort (n = 293 in Yaoundé, Cameroon; n = 29 in Dakar, Senegal; and n = 47 in Bobo-Dioulasso, Burkina Faso) were included (Figure 1). The characteristics of the participants at baseline are presented in Supplementary Tables S1, S2, and S3. The DCU cohort consisted of 73.1% female participants, with median age (interquartile range) of 37 (31.0–43.4) years old, median eGFR of 99.3 (86.1–111.7) ml/min per 1.73 m^2, and CD4 count of 202 (103–342) cells/μl. The proportion of eGFR <60 ml/min per 1.73 m^2 was 1.9%, and that of hypertension was 6%. The median time to starting ART was 1.1 (0.6–5.0) months after the first visit in DCU. The 2LADY cohort consisted of 71.3% female participants, with median age of 38 (33–47) years old, median eGFR of 95.7 (80.9–111.2) ml/min per 1.73 m^2, and CD4 count of 176 (79–288) cells/μl. The proportion of eGFR <60 ml/min per 1.73 m^2 and hypertension were 3.0% and 7.3%, respectively. The median duration of first-line ART exposure was 4.3 (3.0–5.9) years. Overall, the 2LADY cohort participants were older (P < 0.001), had

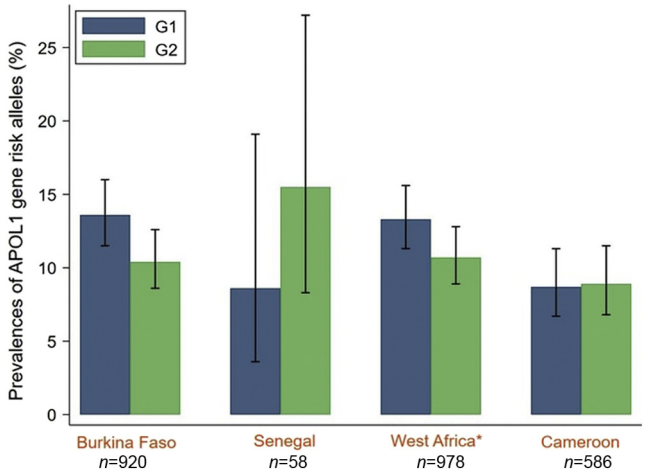

Figure 2. Allelic frequency of *APOL1* risk variants in PLHIV in West Africa and Cameroon. *West Africa = Burkina Faso + Senegal; N, number of alleles (G0, G1, and G2). The bar graph displays the *APOL1* risk alleles' frequency (G1 in blue and G2 in green) with the corresponding 95% CIs. PLHIV, people living with HIV.

lower baseline CD4 count ($P = 0.005$), and lower baseline eGFR ($P = 0.019$) compared with the DCU participants.

Regarding the *APOL1* genetic risk, there was no difference in the characteristic distribution between *APOL1* HR and LR subgroups in both cohorts (Supplementary Tables S2 and S3).

Prevalence of APOL1 Risk Alleles

The Hardy-Weinberg equilibrium was verified for *APOL1* genotype distribution in both cohorts ($P > 0.05$). In DCU, the G1 allele prevalence (95% CI) was 13.7% (11.5–16.2) and 10.8% (8.8–13.1) for the G2 allele (Supplementary Table S4A). In this cohort, 158 (38.3% [33.7–43.1]%) participants carried 1 risk allele and 22 of 413 (5.3% [3.5–8.0]%) carried 2 risk alleles (HR; Table 1). In 2LADY, the G1 and G2 allelic frequencies were 9.2% (7.3–11.5)% and 9.2% (7.3–11.5)%, respectively. The prevalence of *APOL1* HR in the 2LADY cohort was 3.3% (1.9–5.6)% (12 of 369).

When pooling data from both cohorts, the G1 prevalence was significantly higher in the West African sites (Burkina Faso and Senegal) compared with the Central African site in Cameroon (13.3% [11.3–15.6]% vs. 8.7% [6.7–11.3]%, $P = 0.006$) (Figure 2 and Supplementary Table S4A). The G2 allele was also more frequent in West Africa compared with Cameroon, but the difference was not statistically significant (10.7% [8.9–12.8]% vs. 8.9% [6.8–11.5]%, $P = 0.235$). Finally, there were 4.9% [3.3–7.2]% and 3.4% [1.8–6.2]% of the participants who carried the HR genotype in the West African sites and Cameroon, respectively (Table 1 and Supplementary Table S4B).

APOL1 Risk Alleles and Baseline eGFR

In the DCU cohort, where all individuals were ART naive at their first visit, there was no association between *APOL1* HR and eGFR at baseline in the unadjusted analysis or after adjustment on age, hypertension, glycemia, and CD4 count (Table 2). Nevertheless, we found a significant interaction between *APOL1* risk status and the immunosuppression status of the patients as reflected by their CD4 T-cell count level. In the subgroup with low CD4 counts (<200 cells/μl), *APOL1* HR was associated with lower eGFR ($\beta = -21.7$ [−35.1 to −8.3] ml/min per 1.73 m^2, $P = 0.002$) whereas eGFR was not associated with

Table 2. Difference in baseline eGFR and in annual eGFR change according to *APOL1* risk status

	DCU cohort		2LADY cohort	
Baseline and annual eGFR models	**0/1 allele ($n = 391$)**	**2 alleles ($n = 22$)**	**0/1 allele ($n = 357$)**	**2 alleles ($n = 12$)**
Baseline eGFR (ml/min per 1.73 m^2)				
Mean (95% CI)	97.7 [95.9–99.6]	94.0 [85.2–102.8]	94.6 [92.6–96.6]	101.0 [89.5–112.6]
Estimated difference in baseline eGFR, 2 vs. 0/1 alleles (95% CI)[a]				
Unadjusted	Reference	−3.7 [−11.7 to 4.2]	Reference	6.5 [−4.6 to 17.6]
Adjusted on age, HTN, glycemia, and CD4 count	—	−4.2 [−11.9 to 3.4]	—	8.5 [−1.7 to 18.7]
Adjusted on age, HTN, glycemia, and HIV viral load	—	—	—	10.8 [0.8–20.8]
Adjusted on age, HTN, glycemia, CD4 count, and HIV viral load	—	—	—	10.7 [0.8–20.7]
Annual change in eGFR (ml/min per 1.73 m^2)[b]				
Mean (95% CI)	−0.8 [−1.0 to −0.6]	−0.7 [−2.2 to 0.7]	2.0 [1.7–2.4]	1.1 [−0.5 to 2.6]
Estimated difference in eGFR annual change, 2 vs. 0/1 alleles (95% CI)[b]				
Unadjusted	Reference	0.2 [−0.8 to 1.2]	Reference	−1.0 [−3.1 to 1.1]
Adjusted[c] on age, HTN, glycemia, eGFR, and CD4 count	—	0.0 [−1.1 to 1.2]	—	−1.2 [−3.4 to 1.0]
Adjusted[c] on age, HTN, glycemia, eGFR, CD4 count, and HIV viral load	—	—	—	−1.2 [−3.4 to 1.0]

DCU, day care unit; eGFR, estimated glomerular filtration rate; HTN, hypertension.
[a]Linear regressions were used to obtain the coefficients.
[b]Linear mixed model regressions were used to obtain the coefficients.
[c]Adjustment variables were included using values recorded at baseline.
At baseline, there was no difference in mean eGFR between low-risk (0/1 allele) and high-risk (2 alleles) genotypes in the DCU cohort. After adjusting for age, hypertension, glycemia, CD4 count, and HIV viral load, 2LADY patients with high-risk genotype had on average a 10.7 ml/min per 1.73 m^2 higher GFR than those with a low-risk genotype. During follow-up, the annual eGFR change was not significantly different between high-risk and low-risk genotypes in both cohorts.

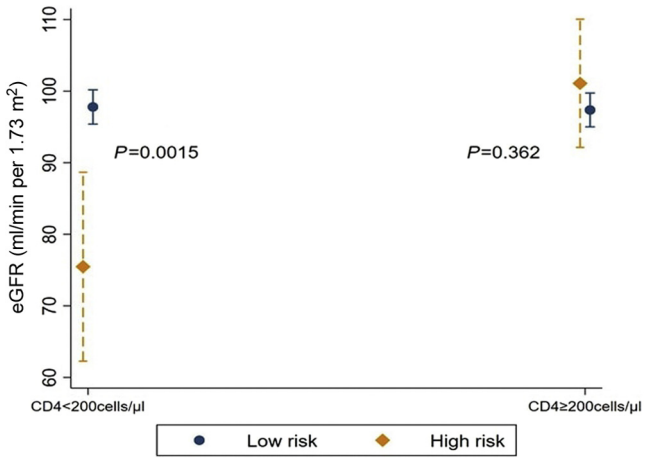

Figure 3. Baseline eGFR by CD4 levels and *APOL1* risk status (DCU cohort). Mean baseline eGFR with the corresponding 95% CIs is presented for the DCU cohort's participants according to baseline CD4 count (<200 cells/µl and ≥200 cells/µl) and to *APOL1* risk status (high-risk [dark orange diamond] and low-risk [navy circle] genotypes). Predicted kidney function values were adjusted for age, glycemia, and hypertension. Mean baseline eGFR was significantly lower in the *APOL1* high-risk group only for patients with low CD4 counts. DCU, day care unit; eGFR, estimated glomerular filtration rate.

APOL1 risk genotype among participants with CD4 count ≥200 cells/µl (Figure 3).

In the 2LADY patients with a long history of ART, baseline eGFR was associated with *APOL1* genotype in an unexpected direction, where patients with HR genotype had a higher mean eGFR than those with LR genotype (β = 10.7 [0.8–20.7] ml/min per 1.73 m^2, P = 0.036 coh) (Table 2). In this ort, at baseline, a higher eGFR was also significantly associated with lower CD4 count and a higher viral load (Supplementary Table S5).

APOL1 Risk Alleles and Changes in eGFR During Follow-Up

The DCU participants were followed for a median duration of 6.1 (interquartile range: 4.2–8.2) years with an average of 1.9 measurements of creatinine per year. In the entire follow-up, the average eGFR decreased by 0.8 [0.6–1.0] ml/min per 1.73 m^2 per year and 14 incident cases of CKD (5.7 [3.4–9.7] per 1000 person-years at risk), including 1 *APOL1* HR genotype carrier, were reported (Supplementary Table S6). Neither the HR genotype, baseline CD4 count, nor the interaction between *APOL1* and baseline CD4 count was associated with changes in eGFR over time.

For the 2LADY participants, the median follow-up on second-line ART was 4.8 (interquartile range: 3.9–5.4) years with 3.1 measurements of creatinine per year on average. Over time, the eGFR increased by 2.0 (1.7–2.3) ml/min per 1.73 m^2 per year and 16 incident cases of CKD (10.3 [6.3–16.8] per 1000 person-years at risk) were reported, all in the *APOL1* LR subgroup (Supplementary Table S6). Neither the HR genotype, baseline CD4 count, nor the interaction between *APOL1* and baseline CD4 count was associated with changes in eGFR. Nevertheless, high baseline HIV viral load (≥5 log/ml) was associated with faster eGFR progression (β = −1.2 [−2.0 to −0.4] ml/min per 1.73 m^2 per year, P = 0.0018). In the subgroup with both high baseline HIV viral load and HR genotype, progression in eGFR was slower (β = −3.9 [−7.7 to −0.1] ml/min per 1.73 m^2 per year, P = 0.046). These results held even after adjusting for baseline eGFR (Figure 4 and Supplementary Tables S7 and S8).

DISCUSSION

Prevalence of APOL1 Risk Alleles

In this report, we described for the first time the *APOL1* renal risk allele frequencies in PLHIV from 3 countries of West (Burkina Faso and Senegal) and Central (Cameroon) Africa. The G1 and G2 allele frequencies were previously reported in various African populations.[31,32] The reported frequencies were highly variable across studies, even within the same country, but the overall geographic distribution indicated higher frequencies of G1, and to a lesser extent of G2 alleles in West Africa.[14,31] This study, reporting higher frequencies for G1 among PLHIV from Burkina Faso and Senegal (13.3%) than among PLHIV from Cameroon (8.7%), confirmed this geographic pattern. Nevertheless, the frequencies reported here in the West African sites were far below most observations within this region, which mainly came from 2 countries, Ghana and Nigeria, where the G1 allele frequencies exceeded 40% in several reports or subpopulations.[31] Outside these 2 countries, there were few data available in West Africa and none in Burkina Faso, to our best knowledge. Further investigation is therefore necessary to better describe and understand the geographic distribution of the *APOL1* risk variants in West African populations. Our data stood in the line of previous reports from Central Africa. We observed *APOL1* risk allele frequencies (G1, 8.7% and G2, 8.9%) close to the previous studies among the general population in Cameroon (G1, 0.8%–16.4% and G2, 3.3%–12.3%)[31] and among HIV-infected children from the Democratic Republic of Congo (G1, 13.5% and G2, 9.6%).[32]

Although the frequency of the *APOL1* HR genotype was estimated at 14% in African Americans, very few data were available in Africa.[14] In a pediatric population from the Democratic Republic of Congo, the HR prevalence was 5.7% in PLHIV and 7% in the general

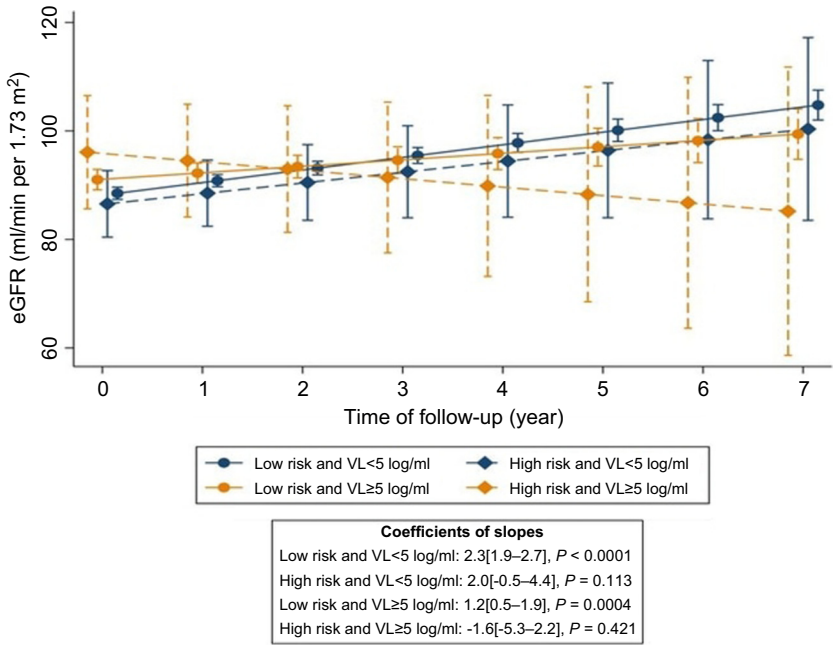

Figure 4. Changes in eGFR over time by baseline HIV VL and *APOL1* risk status (2LADY cohort). Predicted longitudinal eGFR with the corresponding 95% CIs is presented for the 2LADY cohort's participants according to baseline HIV VL (<5 log/ml [navy color] and ≥5 log/ml [dark orange color]) and to *APOL1* risk status (high-risk [diamond and dashed line] and low-risk [circle and solid line]). eGFR annual change was significantly faster in the *APOL1* high-risk group exhibiting high baseline HIV VL. Predicted values were adjusted for the following baseline variables: age, eGFR, glycemia, hypertension, and CD4 count level (see Supplementary Table S8 for further details). eGFR, estimated glomerular filtration rate; VL, viral load.

population.[32] In a recent study on a cohort of HIV-positive Nigerian adults in northern of the country, the HR prevalence was 6.2%, with large variation by ethnic group (Hausa/Fulani = 2.1%, Igbo = 49.1%, and Yoruba = 14.5%).[33] The HR prevalences in Cameroon (3.4%) or West Africa (4.9%) are close to these, but they are much lower than the 23% HR frequency reported in the Igbo group from Nigeria,[30] hence limiting our study power for detecting genetic association. To date, Nigeria and to some extent Ghana look like the exceptions regarding *APOL1* frequencies. Despite studies carried out in several Nigerian ethnic groups (i.e., the Yoruba, Esan, and Igbo), the *APOL1* risk alleles and HR genotype prevalences remain constantly higher than any other tested country.[31,34] The selective pressures responsible for the HR frequencies of G1 and to a lesser extent G2 among certain ethnic groups, primarily residing the eastern coastal regions of West Africa, remain to be elucidated.

APOL1, Immunosuppression Status, and Kidney Function

A few studies in African Americans have reported *APOL1* HR genotype association with progressive loss of kidney function. In the African American Study of Kidney Disease and Hypertension, Parsa *et al.*[35] found a more rapid decline in kidney function in individuals with the HR genotype compared with those with the LR genotype. Similarly, in another cohort study of young to middle-aged adults with preserved kidney function in the USA, HR genotype was associated with faster decline in eGFR by 0.38%.[36] The decline in kidney function in the general population is similar by *APOL1* genotype, but once renal injury occurs (onset of proteinuria), eGFR declines more rapidly in HR Black individuals. Similarly, HR Black individuals had the earliest onset of albuminuria compared with LR Black and White individuals.[36] *APOL1* HR genotypes seem to be specifically associated with renal injury that occurs more frequently and at a younger age.

In contrast with these American studies, we did not find a direct association between *APOL1* HR genotype and decrease in kidney function over time, which could partly stem from unexpected limited study power (n = 22 and 12 HR patients in DCU and 2LADY, respectively). Even more surprising, we observed that the 2LADY cohort participants carrying the HR genotype had, at baseline, a higher eGFR than those with the LR genotype. In addition, we have found that severe immunosuppression (CD4 counts <200 cells/μl) and high HIV viral load (≥5 log/ml) were associated with higher baseline kidney function in the 2LADY cohort. These are unexpected results as most previous studies revealed that advanced HIV disease is accompanied by a deterioration in kidney function.[5,37] Nevertheless, a similar counterintuitive association

was reported in the DART cohort (where participants were symptomatic [World Health Organization disease stage ≥2] with CD4 cell counts <200 cells/μl at ART starting).[38] These authors suggested that this result most likely reflected survivor and enrollment bias, that is, participants with both lower CD4 cell counts and poorer renal function were less likely to survive to meet enrollment criteria. Indeed, kidney impairment is associated with high mortality, both before and during ART.[3–5] The 2LADY trial aimed to compare different second-line regimens among PLHIV failing first-line ART; participants had therefore to survive the pre-ART period and an unsuccessful first-line ART period. In addition, one of the 2LADY inclusion criteria in the trial was creatinine clearance >50 ml/min, which accentuated the selection bias.

Nevertheless, when investigating the interactions between *APOL1* and immunologic or virological status in the 2 HIV cohorts with different study design, the results supported that the deleterious impact of the HR genotype on kidney function could be modulated by the immunologic or the virological status of the patients. In DCU, we found that HR participants initiating ART had lower baseline eGFR than participants with LR genotype, but only among those with CD4 count <200 cells/μl. It should be noted also that ART-naive PLHIV with CD4 count <200 cells/μl most often have very high HIV viral loads.[39,40] Therefore, the observed association may be indirectly related to the virological status of patients. In 2LADY evaluating second-line ART strategies, *APOL1* HR participants with a baseline viral load >5 log/ml had the poorest kidney function progression over time. This association between *APOL1* and kidney function modulated by viral load levels has also previously been reported among HIV-infected men in USA. In that study, *APOL1* HR was associated with a faster eGFR decline over time in men who did not have sustained viral load suppression.[13]

APOL1 and CKD

Many studies investigating the impact of *APOL1* genotype on kidney function have focused on incident clinical outcomes (CKD or ESKD) rather than on the loss of kidney function.[35,41] In addition, a Nigerian study found that HR carriers were associated with a higher occurrence (odds ratio = 4.8) of CKD in the general population.[30] Owing to the unexpected low *APOL1* allele frequency, this study was not powered to evaluate the risk of CKD progression according to the *APOL1* genotype as both the numbers of HR carriers and CKD events were too low. Indeed, no case of CKD was recorded in HR genotype carriers in the 2LADY cohort and only 1 case was recorded in this group in DCU cohort. Yet, the incidence of CKD in the DCU and 2LADY cohorts (5.7 and 10.3 per 1000 person-years, respectively) was consistent with previous studies investigating kidney function among PLHIV in different countries of West Africa.[42]

Limitations of the Study

This study is the first to estimate the prevalence of the *APOL1* HR variants among PLHIV in West Africa, where high allelic frequencies were previously reported in Nigeria and Ghana from the southwest coastal regions. With data from naive and long-term ART-exposed patients from 3 West and Central African cohorts, we were able to explore the association between kidney function and *APOL1* genotype. Robust interpretation of the results was, however, compromised by a lack of statistical power, as the observed *APOL1* HR frequencies in our settings (3.4%–5%) were far below the figures reported in previous studies from West Africa and USA in African Americans (15%–20%) and on which we based our sample size calculation. The 2 cohorts investigated here had different study designs, which strengthens similar conclusions but also makes some comparisons more challenging, as for example the lack of regular baseline viral load measurements in DCU. In addition, we cannot exclude a survivor bias in the study cohorts, when people with both lower immunity condition and poorer renal function would be less likely to survive and meet the study enrollment criteria. Furthermore, the sample size for Cameroon, a country from Central Africa, was smaller than that for the 2 West African countries ($n = 293$ vs. $n = 489$), calling for additional studies in the area. Finally, the study lacked baseline proteinuria data to better characterize kidney function outcomes beyond eGFR.

CONCLUSION

This first study on *APOL1* in West African PLHIV provided a better picture of *APOL1* prevalence in sub-Sahara African PLHIV. It underlined the particularity of Nigeria and Ghana in the region and strongly advocates for the urgent need in larger genetic studies in diverse populations and regions of Africa. Despite limited power, this study suggested a modulation of *APOL1* kidney damage by HIV virological status, but larger cohort studies will be needed to validate this observation.

DISCLOSURE

All the authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

NFK contributed to the data collection and study design, performed statistical analysis, and wrote the manuscript. AP, JZ, EBR, and VD contributed to the data collection, discussion, and reviewed/edited the manuscript. LC, AS, ABS, SED, SKS, CK, NFG, and NM contributed to the discussion and reviewed/edited the manuscript. SL and CW contributed to the study design, genetic analyses, discussion, and reviewed/edited the manuscript. AC contributed to the study design, statistical analysis, discussion, and reviewed/edited the manuscript. All authors have read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study cannot be made publicly available because patients did not consent to public sharing of their data. Access to data requires administrative authorization from the head of the Infectious Diseases Department of Souro Sanou University Hospite and the 2LADY/ANRS-12169 Scientific comity, after evaluation of the request. Researchers who wish to access some of the data from this study must address a request detailing the types of analyses they wish to perform. This request can be sent directly to the corresponding author at: sophie.limou@univ-nantes.fr; telephone: +33 244 768 271.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study, which involves participants from Cameroon, Burkina Faso, and Senegal, has been approved by the ethics committees of the 3 respective countries' ("Comité d'éthique pour la recherche en santé" for Burkina Faso, "Comité national d'éthique pour la recherche en santé" for Senegal, and "Comité national d'éthique de la recherche pour la santé humaine" for Cameroon) upstream project initiation. Written consent was obtained from participants, and a waiver of consent was obtained from the ethics committee for participants in the 2LADY trial who were no longer alive at the time of this study.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. Baseline characteristics of all participants.

Table S2. Baseline characteristics of the day care unit (DCU) cohort participants stratified by *APOL1* risk status.

Table S3. Baseline characteristics of the 2LADY cohort participants stratified by *APOL1* risk status.

Table S4A. Prevalences of *APOL1* risk alleles.

Table S4B. Distribution of *APOL1* genotypes.

Table S5. Factors associated with eGFR in the 2LADY cohort at baseline.

Table S6. Follow-up data by study cohort.

Table S7. Baseline predictors of eGFR annual change in the 2LADY cohort.

Table S8. Baseline predictors of eGFR annual change in the 2LADY cohort by stratifying with the interaction between *APOL1* and baseline HIV viral load.

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