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APOL1 Genotype and HIV Infection: 20-Year Outcomes for CKD, Cardiovascular Disease, and Hypertension

Katherine K. Tassiopoulos[1], Kunling Wu[2], Zhenzhen Wu[3], Edgar T. Overton[4,5], Frank J. Palella[6], Christina Wyatt[7], Robert C. Kalayjian[8] and Leslie A. Bruggeman[3,9]

[1]Department of Epidemiology, Harvard T. H. Chan School of Public Health, Boston, Massachusetts, USA; [2]Center for Biostatistics in AIDS Research, Harvard T. H. Chan School of Public Health, Boston, Massachusetts, USA; [3]Department of Inflammation & Immunity, Cleveland Clinic, Cleveland, Ohio, USA; [4]Division of Infectious Diseases, Department of Medicine, University of Alabama at Birmingham, Birmingham, Alabama, USA; [5]ViiV Healthcare Medical Affairs, Durham, North Carolina, USA; [6]Division of Infectious Diseases, Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA; [7]Division of Nephrology, Department of Medicine, Duke University School of Medicine, Durham, North Carolina, USA; [8]Division of Infectious Diseases, Department of Medicine, MetroHealth Medical Center, Cleveland, Ohio, USA; and [9]Department of Kidney Medicine, Cleveland Clinic, Cleveland, Ohio, USA

Introduction: *APOL1* variant alleles substantially increase the risk for chronic kidney disease (CKD) in Black individuals, especially in the setting of HIV infection; however, their impact on hypertension and cardiovascular disease (CVD) is unclear.

Methods: Black persons with HIV ($n = 1194$) followed in the AIDS Clinical Trials Group (ACTG) observational studies A5001 and A5322 were genotyped for *APOL1* risk alleles. Cox proportional hazard models were used to assess associations between *APOL1* genotype and incident CKD, CVD, and hypertension, and linear mixed effects models were used to examine associations with longitudinal estimated glomerular filtration rate (eGFR) and proteinuria. Plasma HIV-1 viral suppression was evaluated as an effect modifier.

Results: *APOL1* genotype was associated with CKD, but not with hypertension or CVD, although CVD events were infrequent in this relatively young cohort. Annual rates of eGFR decline and proteinuria were greater among persons with *APOL1* risk alleles, including a detrimental effect of 1 *APOL1* risk allele, which only became evident in the second decade of follow-up. Sustained HIV-1 viral suppression did not alter the association between incident CKD and *APOL1* genotype; however, it was associated with a slower rate of eGFR decline and less proteinuria in participants with at least 1 *APOL1* risk allele, including individuals with eGFRs above the CKD threshold throughout follow-up.

Conclusion: Among treated persons with HIV, *APOL1* risk alleles were associated with CKD and eGFR decline, including an effect of 1 *APOL1* risk allele which took longer to manifest and was greater in individuals who did not achieve sustained viral suppression. Conversely, no association between *APOL1* risk alleles and incident hypertension or CVD was detected.

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Polymorphisms in the gene for Apolipoprotein L1 (i.e., *APOL1*) are common in African Americans and Blacks of sub-Saharan West African ancestry and contribute significant risk for some forms of CKD.[1,2] This relationship is highly relevant in the setting of HIV infection, because HIV-associated nephropathy (HIVAN) is the kidney disease that has been most strongly linked with variant *APOL1* alleles (referred to as the G1 and G2 risk alleles). This CKD risk is recessive, and individuals carrying 2 *APOL1* risk alleles have a 29- to 89-fold increased risk of developing HIVAN.[3,4] Independent of HIV infection, recessive inheritance of G1 and G2 risk alleles is also associated with a 7- to 10-fold increased risk for hypertension-attributed CKD[5–7] and a 2- to 4-fold increased risk for the hypertension-associated pregnancy complication

Correspondence: Leslie Bruggeman, Department of Inflammation & Immunity, Lerner Research Institute. Cleveland Clinic, 9500 Euclid Ave, Cleveland, Ohio, USA. E-mail: bruggel@ccf.org; or Robert Kalayjian, Department of Medicine, MetroHealth Medical Center, 2500 MetroHealth Dr, Cleveland, Ohio 44109, USA. E-mail: rck4@case.edu

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of preeclampsia.[8,9] However, a causal link between *APOL1* risk alleles and hypertension has not been clearly established. There are conflicting reports associating elevated blood pressure with *APOL1* risk alleles, including differences in responsiveness to antihypertensive treatments based on *APOL1* genotype.[6,10-12] In the setting of HIV infection, the relationship between hypertension and *APOL1* risk alleles has not been previously examined.

Several studies have explored the relationship between *APOL1* risk alleles and CVD.[13-20] These previous studies utilized large multiinstitutional cohorts designed to examine CVD and CKD outcomes but reported inconsistent results. A meta-analysis incorporating findings from several previous studies and a recent analysis including over 30,000 Black participants failed to identify an association between *APOL1* genotype and CVD risk that was independent of comorbid CKD.[21,22] To date, no study has reported on the relationship between *APOL1* genotype and CVD risk in persons with HIV.

Leveraging up to 20 years of follow-up in multicenter ACTG longitudinal observational studies, we evaluated the association between *APOL1* risk alleles and hypertension, CVD, and CKD over long-term follow-up.

METHODS

Participants and Study Design

Study participants were self-identified Black adults (regardless of Hispanic ethnicity) who were enrolled in the ACTG long-term observational study A5001[23] (known as ACTG Longitudinal Linked Randomized Trials [ALLRT]) with many participants continuing follow-up into the long-term observational study A5322[24] (known as HIV Infection, Aging, and Immune Function Long-Term Observational Study [HAILO]). ALLRT participants were enrolled between 1999 and 2011 with follow-up ending in 2013; enrollment into HAILO took place between 2013 and 2014 and follow-up ended in 2021. Participants followed in ALLRT that continued follow-up in HAILO had continuity in care and observation, and all HAILO participants had been previously followed in ALLRT. All participants received initial antiretroviral therapy (ART) as part of randomized ACTG clinical trials (parent trials). All participants provided written informed consent, and each participating ACTG study site received approval from its designated institutional review board. Only participants who provided informed consent via ACTG A5128[25] for use of their stored biospecimens in unspecified or genetic analyses were included in this study. Sex was defined as male or female identity assigned at birth and was use for all analyses. Evaluations included physical exams with blood and urine collections at 4 to 12-month (A5001) or 6 to 12-month (A5322) intervals; and medical chart abstraction for diagnoses, medications, and laboratory test results, including kidney function tests. The baseline for these analyses was defined as entry into the parent trial or the first study visit in which kidney function was measured.

Outcomes

Hypertension was defined as systolic pressures $\geq$ 140 mm Hg or diastolic pressures $\geq$ 90 mm Hg at 2 consecutive visits, a documented hypertension diagnosis, or use of antihypertensive medication. CVD was a composite outcome defined as acute myocardial infarction, congestive heart failure, stroke, undergoing a revascularization procedure, or a CVD-attributed death. eGFR was calculated using the race neutral CKD-Epidemiology Collaboration 2021 equation.[26] CKD was defined as eGFR $<$ 60 ml/min per 1.73 m^2 or proteinuria with urinary protein-to-creatinine ratios (UPCR) $\geq$ 0.2 mg/mg. Prevalent CKD was defined as a study entry eGFR that was $<$ 60 ml/min per 1.73 m^2. Owing to limitations in the collection of proteinuria data in some parent trials (use of dipstick or reporting only nephrotic range values), the prevalent CKD analysis was based on eGFR criteria alone. The incident CKD analysis included participants who met either the eGFR or UPCR criteria but required repeat measurements (eGFR, 2 readings $\geq$ 3 months apart within a 12-month period; and proteinuria, 2 readings $\geq$ 3 months). Incident CKD analyses excluded participants with prevalent CKD and excluded participants that experienced a single UPCR measurement exceeding the CKD definition to avoid potentially misclassifying participants with CKD in the control group (equivocal for case/control designation). Potential causes of transient proteinuria such as urogenital infections or substance abuse were not tracked in ALLRT or HAILO. UPCR was $\log_{10}$-transformed because it was not normally distributed. Both eGFR and UPCR were also evaluated as continuous measures of kidney function.

Effect Modifiers

HIV-1 viral suppression status was examined (using recommended methods for viral load tracking[27,28]) as the percent of time under observation spent suppressed, with HIV-RNA $<$ 200 copies/ml on $\geq$ 90% of measurements considered sustained viral suppression, versus $<$ 90% of measurements and designated as nonsustained viral suppression. Although the parent trials used different ART regimens, the key metric for this study was achieving viral suppression, which was independent of treatment modality.

APOL1 Genotyping

Participants were genotyped using a TaqMan allelic discrimination assay (ThermoFisher Scientific, Waltham, MA) for the *APOL1* G1 and G2 risk alleles, which has been validated for human studies.[29] Genotyping included the single nucleotide polymorphisms rs73885319 (assay ID-AH20SD1) and rs60910145 (assay ID-AHWR1JA) that define the G1 allele, and rs71785313 (assay ID-AH1RT7T) that defines the G2 allele. Peripheral blood mononuclear cell pellets were obtained from the ACTG biorepository and genomic DNA was extracted using the KingFisher Flex Purification system in 96-well format with solid phase reversible immobilization (Sera-Mag Speed beads, ThermoFisher Scientific, Waltham, MA). DNA was diluted with water to the 1 to 20 μg/ml range and 2 μl of diluted DNA was air-dried overnight on 384-well plates. TaqPathProAmp Master Mix (Thermo Fisher Scientific, Waltham, MA) was mixed with the individual TaqMan assay and added to wells at a final volume of 5μl. Amplification was on the QuantStudio5 thermocycler (Applied Biosystems, ThermoFisher Scientific, Waltham, MA) using the recommended genotyping amplification protocol (hot start with 40 cycles of 95 °C for 15 s, 60 °C for 1 min).

Statistical Analyses

The primary exposure variable was carriage of the *APOL1* G1 and G2 risk alleles, examined in dominant, additive, and recessive inheritance patterns. Associations with *APOL1* risk alleles and incident disease outcomes were first graphically presented with Kaplan–Meier plots. Time-to-event was censored at the first occurrence of either the disease outcome, death, date of last study visit, or loss to follow-up. Locally estimated scatterplot smoothing plots were prepared for eGFR and UPCR over time by number of *APOL1* risk alleles, overall and stratified by time-updated viral suppression status. Cox proportional hazards models were fit to estimate the association of *APOL1* risk alleles with each incident disease outcome. Participants with prevalent disease were excluded from incident analyses. For the incident CVD analysis, prevalent disease exclusions also included a history of angina pectoris, coronary artery disease, and peripheral artery disease ($n = 3$), or CKD ($n = 11$) at baseline. For incident hypertension analysis, participants with CKD at baseline ($n = 7$) were excluded. The proportionality assumption was tested by including interaction terms of *APOL1* risk alleles as a function of survival time. A competing risk analysis for death was examined using 2 regression models (Fine and Gray's method and cause-specific method), which generated similar results and *P*-values.

Linear mixed effect models were fit to estimate the associations between *APOL1* genotype and UPCR and eGFR over time. These models included *APOL1*, time, an interaction term between time and *APOL1* genotype as a fixed effect, and the intercept as a random effect. Variables that were known risk factors for the disease outcomes were evaluated in the above models as confounders. The list of potential confounders, both those considered at baseline and variables time-updated in 16-week intervals, are listed in Supplementary Table S1. Each potential confounder was included individually in the univariable model and those that changed the univariable effect estimate of *APOL1* genotype on the disease outcome by ≥ 10% were included in the final multivariable model. Time-updated viral suppression status was evaluated as an effect modifier by including an interaction term between the effect modifier and *APOL1* genotype in the multivariable models. All analyses were conducted with SAS v9.4 (SAS Institute Inc. 2023, SAS/STAT 15.3 User Guide, Cary, NC).

RESULTS

Of 1647 participants who self-reported as Black, 415 did not consent to the use of their specimens or did not have available specimens, 27 were equivocal for case or control designation, and 11 did not have *APOL1* genotyping. With these exclusions, the study group included 1194 participants, 71% of whom were male sex at birth (gender identity was not available), with a median age at enrollment of 36 years (Table 1). All participants were ART-naive at study entry, and

Table 1. Demographics and clinical characteristics at baseline ($n = 1194$)

Characteristic	*n* (%) or median	IQR
Male sex	847 (71%)	
Age, yrs	36	29–46
Hispanic ethnicity	40 (3%)	
Body mass index, kg/m^2	25	21.9–29.0
Prior smoker	375 (31%)	
Current smoker	292 (24%)	
History of diabetes	65 (5%)	
History of hypertension	317 (27%)	
History of CVD	19 (2%)	
History of CKD	13 (1%)	
eGFR, ml/min per 1.73 m^2	103	87–115
UPCR, mg/mg	0.092	0.060–0.173
Statin use	31 (3%)	
Fasting LDL, mg/dl	94	76–116
Tenofovir disoproxil fumarate use	603 (52%)	
CD4 count, cell/mm^3	265	117–405
HIV RNA viral load, copies/ml	27,960	5209–80,964

CKD, chronic kidney disease; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; IQR, interquartile range; LDL, low-density lipoprotein; UPCR, urine protein-to-creatinine ratio.

median follow-up times and event rates for each comparison group are in presented in Supplementary Table S2. Baseline CD4 count was < 350 cells/mm^3 for 66% of the cohort and 66% had a baseline plasma HIV RNA ≥ 10,000 copies/ml. Only half (46%) achieved sustained viral suppression during follow-up. Use of antiretrovirals with potential kidney or cardiovascular side effects including tenofovir disoproxil fumarate (TDF) and abacavir was common (52% and 23%, respectively). Overall kidney function at baseline was within normal ranges with baseline median eGFR of 103 ml/min per 1.73 m^2 (IQR: 87–115) and median UPCR of 0.092 mg/mg (IQR: 0.06–0.17). Hypertension was prevalent in 27% of participants, and 2% and 1% had a history of CVD and CKD, respectively at baseline.

APOL1 high risk genotypes, consisting of any combination of 2 *APOL1* risk alleles, were present in 11% of the study cohort, which is consistent with the reported African American population frequency.[30] The *APOL1* genotype in the remaining participants was 45% with 1 risk allele and 44% with no *APOL1* risk alleles, and this distribution of genotypes was consistent in subgroup analyses for each of the individual disease outcomes (Supplementary Table S3).

Association With Hypertension

Hypertension was prevalent in 317 participants but was not associated with *APOL1* genotype in any inheritance model (Supplementary Table S4). Among the 870 participants without prevalent hypertension or CKD at baseline, 267 (31%) developed hypertension during follow-up (Supplementary Table S5). Of the 267 incident hypertension cases, 54 (20%) also developed CKD. Participants with incident hypertension were older at baseline, had higher body mass indexes, and were more likely to have diabetes and to have received TDF as part of their initial ART regimen compared to those who did not develop hypertension. There was no association of *APOL1* risk genotypes with incident hypertension under any inheritance model (Figure 1a). Kaplan–Meier curves identified a higher incidence of hypertension during the first 5 years of follow-up in individuals with 2 *APOL1* risk alleles; however, there was no difference in incidence of hypertension by *APOL1* genotype with longer follow-up (Figure 2a). There was no evidence of effect modification by viral suppression (interaction $P \geq 0.4$ for all inheritance models). Death was not a competing risk factor in survival estimates (Supplementary Figure S1A; $P \geq 0.3$ for all inheritance models).

Association With CVD

CVD was prevalent in 19 participants but was not associated with *APOL1* genotype in any inheritance model (Supplementary Table S4). There were 31 (2.7%) incident CVD cases among 1160 participants without prevalent CVD. Participants with incident CVD were older by a median of 12 years and were more likely to have a history of hypertension and a family history of CVD compared to those without CVD (Supplementary Table S6). Cox proportional hazard models did not demonstrate an association between *APOL1* risk alleles and incident CVD under any inheritance model (Figure 1b). Survival probabilities were not different by number of *APOL1* risk alleles (Figure 2b). No evidence of effect modification by viral suppression was identified (interaction $P \geq 0.8$ for all inheritance models). Death was not a competing risk factor in survival estimates (Supplementary Figure S1B; $P \geq 0.2$ for all inheritance models).

Association With CKD

Prevalent CKD ($n = 13$) was associated with carriage of 2 *APOL1* risk alleles that resulted in associations in dominant, additive, and recessive inheritance models, with an adjusted odds ratio of 7.3 (95% confidence interval [CI] 2.22, 24.11, $P = 0.001$) in the recessive model (Supplementary Table S4). Incident CKD was diagnosed in 187 (20.0%) of the 937 participants without prevalent CKD, either by reaching the proteinuria criterium ($n = 107$), the eGFR criterium ($n = 45$), or both criteria ($n = 35$). Participants with incident CKD were older and included those who were more likely to have a history of diabetes or hypertension or to have received TDF in their initial ART regimen, among other characteristics (Supplementary Table S7). Cox proportional hazards models identified an association (hazard ratio [HR] = 1.55, 95% CI = 1.03–2.33, $P = 0.03$) between *APOL1* risk alleles and incident CKD using a recessive model of inheritance (Figure 1c). The association between *APOL1* risk alleles and incident CKD remained significant after adjusting for initial TDF exposure, or when death was treated as a competing risk factor in cumulative incidence hazard models (Supplementary Figure S1C) when examined as both a recessive trait (HR = 1.57, 95% CI = 1.04–2.35, $P = 0.03$) or an additive trait (2 vs. 0 risk alleles, HR = 1.60, 95% CI = 1.03–2.47, $P = 0.04$). However, this association was no longer significant when adjusted for differences in baseline eGFR in both Cox proportional hazard models (Figure 1c) and cumulative incidence hazard models (recessive: HR = 1.2, 95% CI = 0.75–1.94, $P = 0.5$; additive: HR = 1.18, 95% CI = 0.72–1.95, $P = 0.5$). CKD-free survival probabilities identified an effect of 2 *APOL1* risk alleles in the incident

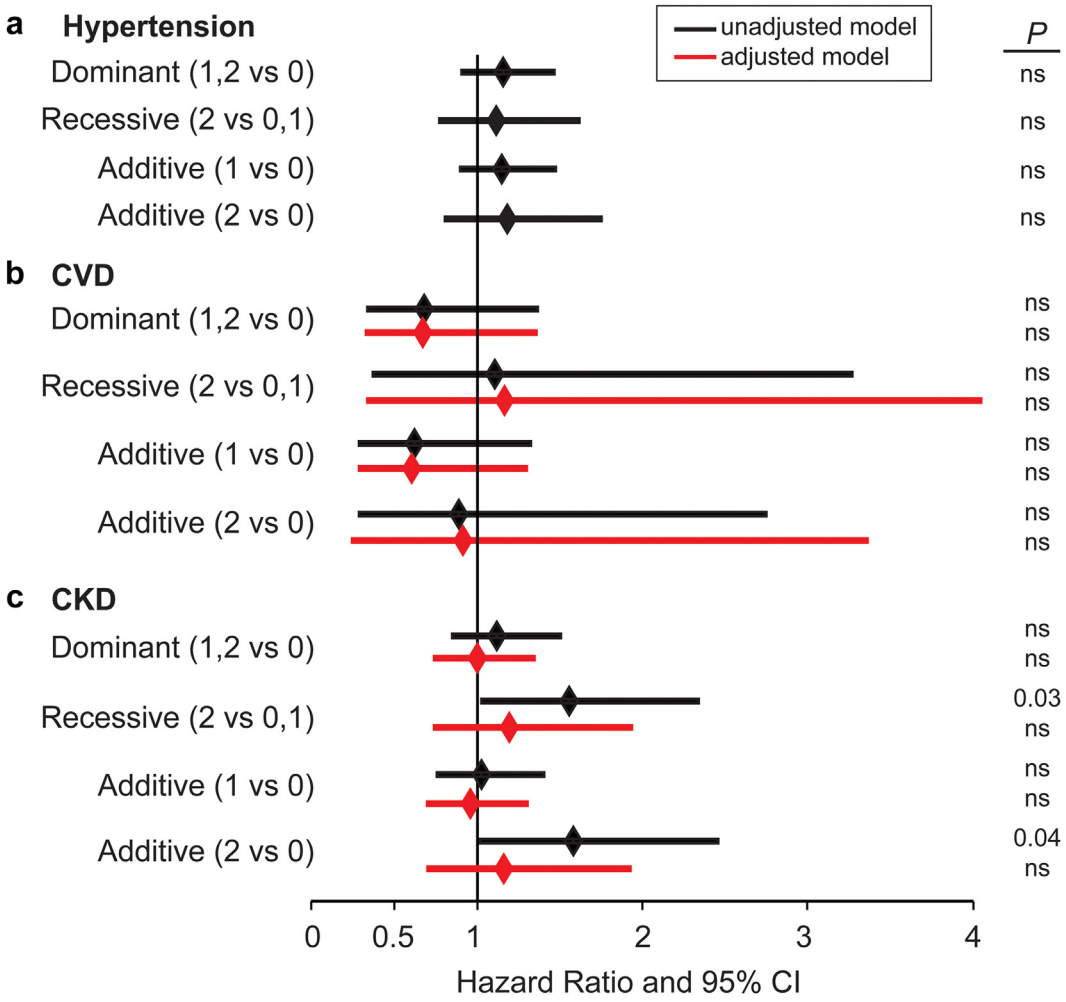

Figure 1. Incident CKD but not CVD or hypertension was associated with 2 *APOL1* risk alleles. Forest plots for association between *APOL1* risk alleles and incident disease in unadjusted and adjusted models for each inheritance model. (a) Incident hypertension ($n = 267$) was not impacted by *APOL1* genotype under any inheritance model. An adjusted model is not presented for the hypertension outcome because there were no variables that changed the association by $> 10\%$. (b) Incident CVD ($n = 31$) was not impacted by *APOL1* genotype under any inheritance model. The adjusted model included smoking history, eGFR, and hypertension. (c) Incident CKD ($n = 187$) was significantly associated with both recessive and additive inheritance of 2 *APOL1* risk alleles but was not significant in the adjusted model. Adjusted model included baseline eGFR. CI, confidence interval; CKD, chronic kidney disease; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; ns, not significant.

CKD cohort (Figure 2c). There was no evidence of effect modification by sustained viral suppression on incident CKD (interaction $P \geq 0.3$ for all inheritance models).

Longitudinal Changes in eGFR and Proteinuria

The potential contribution of 1 *APOL1* risk allele was evident when eGFR and proteinuria were examined longitudinally. Annual eGFR declined by a rate of −1.16 ml/min/yr in persons with either 1 or 2 *APOL1* risk alleles (dominant model) compared to a rate of −0.97 ml/min/yr among those with no *APOL1* risk alleles, with a slope difference of −0.19 ml/min/yr ($P = 0.002$, Table 2). There was effect modification by HIV viral suppression status on eGFR slope in the dominant inheritance model, with differences in annual declines of −0.31 ml/min for nonsustained viral suppression versus −0.08 ml/min for sustained viral suppression (interaction $P = 0.07$). When examining this effect in the recessive model, there were no significant differences according to viral suppression status in eGFR slopes for 0 or 1 risk allele versus 2 risk alleles (slope difference: −0.09 ml/min), suggesting that this effect of viral suppression was driven by a significant preservation of eGFR in participants with 1 risk allele. Participants with 2 risk alleles had greater annual eGFR declines compared to 0 risk allele participants, with no differences based on viral suppression status.

Graphing eGFR trajectories similarly demonstrated modifying effects of HIV viral suppression status on the association of *APOL1* genotype with eGFR. At baseline, participants with 2 *APOL1* risk alleles had lower eGFRs compared to those with 0 or 1 risk alleles

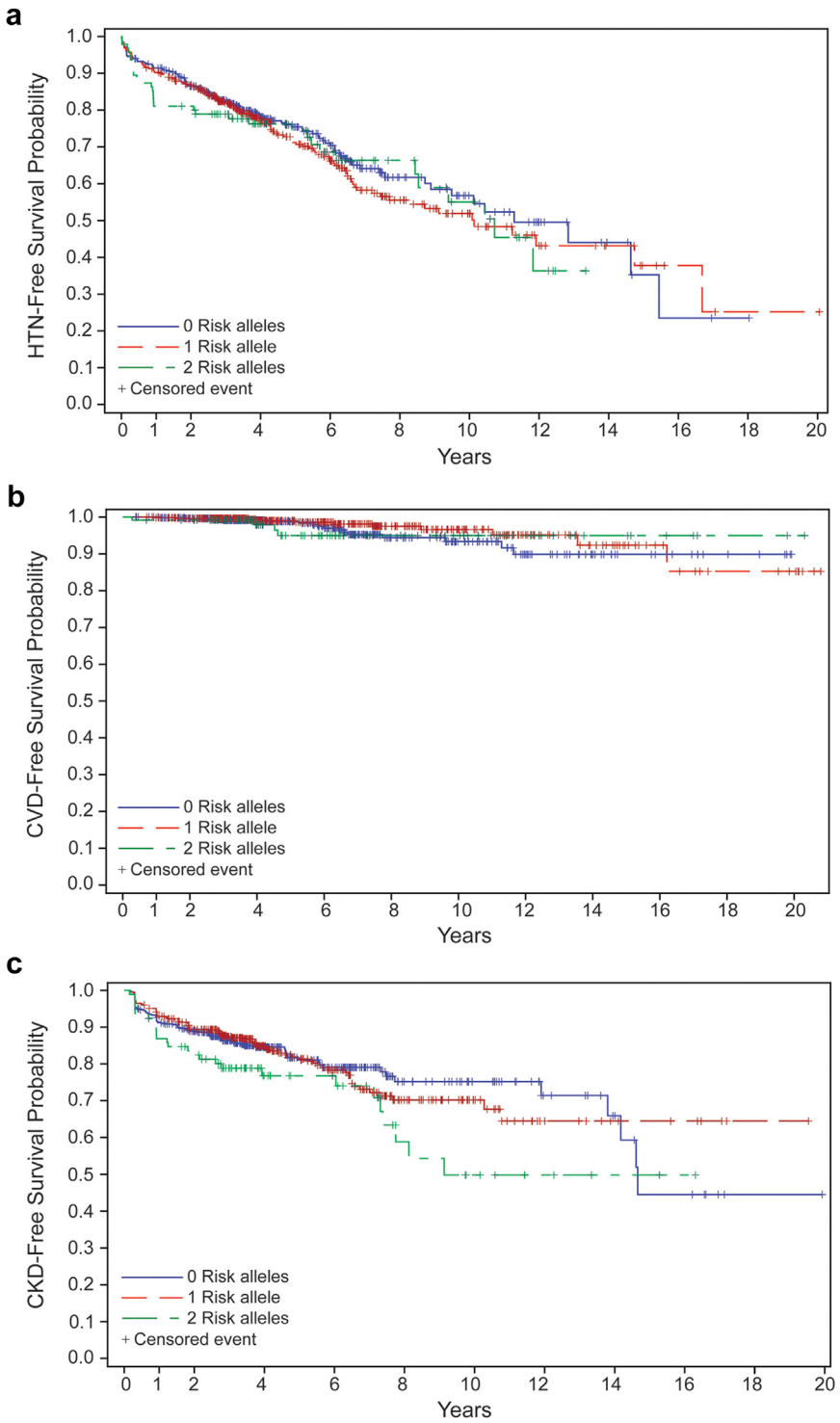

Figure 2. *APOL1* risk alleles associated with CKD, but not CVD or hypertension (HTN). Kaplan-Meier plots of disease-free survival probabilities stratified by number of *APOL1* risk alleles. (a) Incident hypertension ($n = 267$). (b) Incident CVD ($n = 31$). (c) Incident CKD ($n = 187$). Events are censored as defined in the methods. Follow-up in years reflect time since analysis baseline (ART initiation). ART, antiretroviral therapy; CKD, chronic kidney disease; CVD, cardiovascular disease.

in both the sustained and nonsustained viral suppression groups (Figure 3a). There was no difference in age at ART initiation (study entry) that could account for the differences in baseline eGFR (Supplementary Table S8). In the nonsustained suppression group, participants with 1 or 2 risk alleles had faster declines in eGFR than those with 0 risk alleles, which became evident in the second decade of follow-up. In the sustained viral suppression group, the mean eGFR of participants with 2 *APOL1* risk alleles improved over the first 5 years of treatment, possibly reflecting participants in early stages of HIVAN that initially

Table 2. Annual slope change in eGFR and proteinuria by *APOL1* risk alleles

Cohort	*APOL1*	eGFR change ml/min per 1.73 m^2	95% CI	*P*	log_{10} UPCR fold change	95% CI	*P*
All participants	0 risk alleles	−0.97	−1.06 to −0.87	<0.001	1.00	0.99–1.01	0.6
	1 risk allele	−1.16	−1.25 to−1.07	<0.001	1.01	1.00–1.02	0.002
	2 risk alleles	−1.16	−1.33 to−0.99	<0.001	1.01	0.99–1.02	0.5
slope difference:	dominant model	−0.19	−0.32 to−0.07	0.002	1.01	1.00–1.02	0.1
slope difference:	recessive model	−0.09	−0.27 to−0.09	0.3	1.00	0.98–1.01	0.7
Sustained viral suppression	0 risk alleles	−0.94	−1.06 to−0.83	<0.001	1.01	0.99–1.02	0.3
	1 risk allele	−0.98	−1.10 to−0.86	<0.001	1.01	1.00–1.02	0.2
	2 risk alleles	−1.18	−1.40 to−0.97	<0.001	1.00	0.98–1.02	0.9
slope difference:	dominant model	−0.08	−0.24 to 0.08	0.3	1.00	0.99–1.02	0.9
slope difference:	recessive model	−0.22	−0.46 to 0.01	0.06	1.00	0.97–1.02	0.7
Nonsustained viral suppression	0 risk alleles	−1.01	−1.16 to−0.85	<0.001	0.99	0.98–1.01	0.5
	1 risk allele	−1.36	−1.49 to−1.24	<0.001	1.02	1.01–1.03	0.002
	2 risk alleles	−1.13	−1.39 to−0.86	<0.001	1.01	0.99–1.04	0.4
slope difference:	dominant model	−0.31	−0.51 to−0.12	0.002	1.02	1.01–1.04	0.01
slope difference:	recessive model	0.10	−0.19 to 0.38	0.5	1.00	0.97–1.03	0.9

CI, confidence interval; eGFR, estimated glomerular filtration rate; UPCR, urine protein-to-creatinine ratio.

improved when started on ART, but subsequently resumed an annual rate of decline. Throughout follow-up, participants with 1 risk allele and sustained viral suppression had a mean eGFR below those with 0 risk alleles, but higher than those with 2 risk alleles. It is noteworthy that in the sustained viral suppression group, mean eGFR remained above the CKD threshold of 60 ml/min per 1.73 m^2 regardless of the number of *APOL1* risk alleles, with only 1 individual with an eGFR below 15 ml/min per 1.73 m^2 indicative of end-stage kidney disease. The results were distinctly different in the nonsustained viral suppression group, in which the eGFR thresholds for both CKD and end-stage kidney disease were reached more frequently (P=0.05 for the difference in number of participants reaching eGFR $<$ 60 or $<$ 15ml/min per 1.73 m^2 based on viral suppression status).

Similarly, more severe proteinuria or more frequent proteinuria onset was observed in participants with *APOL1* risk alleles in the nonsustained viral suppression group (Figure 3b). As with the eGFR trajectories, these differences became evident after approximately 10 years of follow-up. The effect of *APOL1* genotype on proteinuria, however, was eliminated with sustained viral suppression.

DISCUSSION

The current disease paradigm for APOL1 nephropathy is a 2-hit mechanism requiring the *APOL1* genetic susceptibility combined with an environmental stressor, such as HIV infection. In HIVAN, when HIV viral suppression is controlled by ART, the second hit stressor of HIV viremia is mitigated. Thus, with the implementation of suppressive ART, individuals with undetectable viral loads seldom develop HIVAN and the incidence of HIVAN has declined.[31,32] Although some antiretrovirals have nephrotoxic side effects,[33] ART improves kidney function and reduces progression to CKD.[34–36] However, despite sustained viral suppression, we determined that annual rates of eGFR decline were still greater for participants with *APOL1* risk alleles over long-term follow-up. In normal aging, eGFR decline is approximately 1 ml/min/yr and this decline typically starts in the third decade of life.[37] Considering that our cohort's median age at entry was 36 years, we would expect to observe eGFR declines due to normal aging. The observed eGFR slopes for participants with 0 risk alleles approximated the normal aging rate. Declines in eGFR for participants with 1 or 2 risk alleles were greater but did not exceed 3 ml/min/yr, a level that would normally raise concerns of an ongoing CKD process. By the end of follow-up, mean eGFR slopes in the sustained viral suppression group did not decline below the CKD threshold of 60 ml/min per 1.73 m^2 regardless of *APOL1* genotype. However, mean eGFR slopes for participants with both 1 and 2 risk alleles at the end of follow-up were significantly lower than for those with 0 risk alleles. Therefore, sustained viral suppression reduced but did not eliminate the effect of *APOL1* risk alleles on eGFR decline. This effect modification by sustained viral suppression on eGFR is consistent with a previous analysis.[38] Conversely, proteinuria was largely prevented by sustained viral suppression regardless of *APOL1* genotype.

The *APOL1* genetic susceptibility is most strongly apparent when inherited as a recessive trait requiring 2 *APOL1* risk alleles. We identified a significant risk for incident CKD in a recessive inheritance model with an HR that was comparable to HRs reported in the general (HIV uninfected) Black population.[3,39–43] Although the

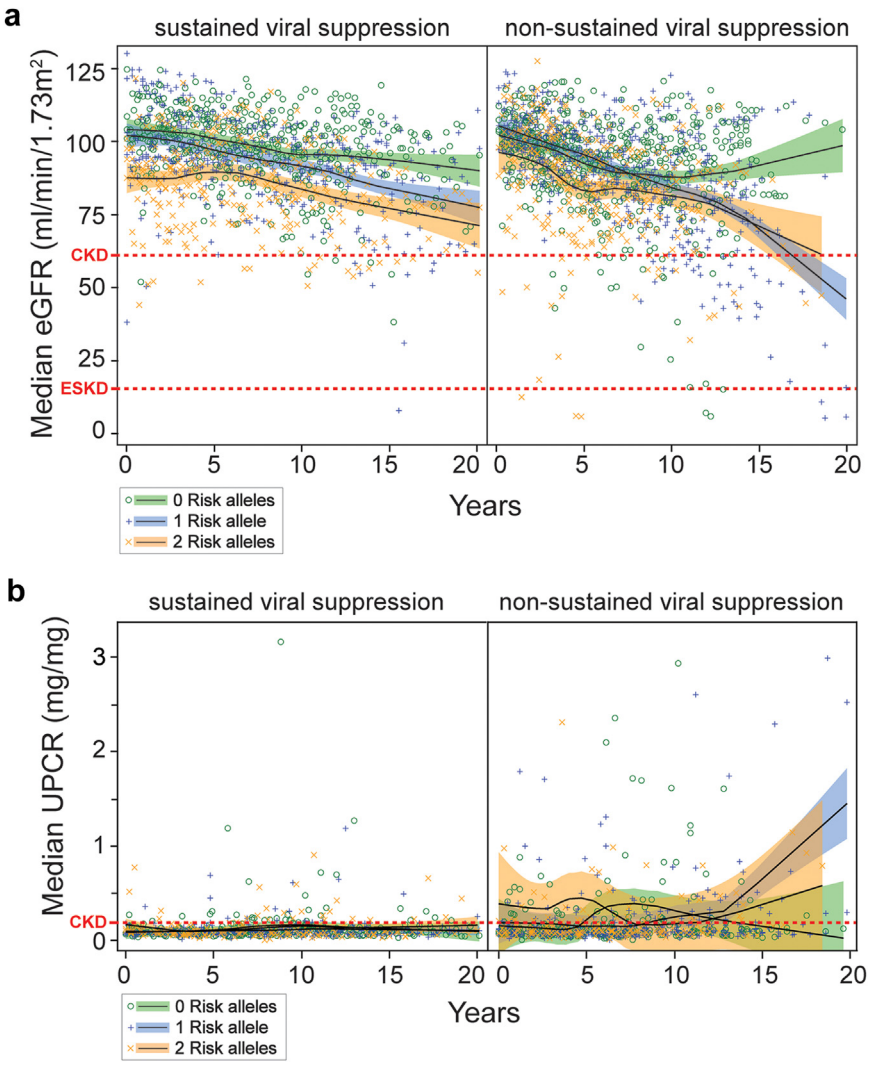

Figure 3. Renal function decline associated with number of *APOL1* risk alleles but was reduced with sustained viral suppression. In all study participants, LOESS curves are shown with mean line and 95% confidence interval bands for longitudinal eGFR (a) and UPCR (b). Curves show comparisons of participants with sustained viral suppression (≥ 90% of viral load assays under 200 HIV-RNA copies/ml, $n = 549$) to those with nonsustained suppression ($n = 645$) and are stratified by number of *APOL1* RA. Thresholds for CKD (eGFR < 60 ml/min per 1.73 m^2 or UPCR ≥ 0.2 mg/mg) and (ESKD, eGFR < 15 ml/min per 1.73 m^2) are marked with red dashed lines. All available measurement time points for each participant are shown. Follow-up in years reflected time since analysis baseline (ART initiation). ART, antiretroviral therapy; CKD, chronic kidney disease; ESKD, end-stage kidney disease; eGFR, estimated glomerular filtration rate; HIV-RNA, human immunodeficiency virus-RNA; LOESS, locally estimated scatterplot smoothing; RA, risk alleles; UPCR, urinary protein-to-creatinine ratio.

major effect of 2 risk alleles has been well-documented, some studies have reported lesser effects of 1 *APOL1* risk allele. In the original study describing *APOL1* genotype effects on HIVAN prevalence, an effect of 1 risk allele was observed although with a smaller effect size with an odds ratio of 1.9 (95% CI: 1.01–3.5) compared to an odds ratio of 29 (95% CI: 13–68) with 2 risk alleles.[3] Interestingly, we also observed an effect of 1 *APOL1* risk allele on eGFR decline, in which the accelerated decline became apparent in the second decade of follow-up and was more evident in participants without sustained viral suppression. One possible conclusion is that carriage of 1 *APOL1* risk allele may also have a negative impact on kidney function as does 2 *APOL1* risk alleles. Mechanistically, how *APOL1* risk variants cause CKD is not fully understood. A leading theory is that risk variants are gain-of-function mutations that have acquired a detrimental function causing cytotoxicity to kidney cells.[30] If this were the case, it remains unexplained why CKD is a recessive trait requiring the inheritance of 2 risk alleles, because individuals with only 1 risk allele would still express a cytotoxic APOL1. Our observations here may provide some clarity on this point, because it appears 1 *APOL1* risk allele does exhibit a detrimental effect on eGFR. This 1 risk allele effect, however, appears to require years of continued exposure to the inducing stressor before progression to a

discernable disease outcome. This observation may have been possible from the unique characteristics of this study, because viral infections are known stressors for APOL1 nephropathy and the cohort included a high percentage of participants without sustained viral suppression during a prolonged follow-up.

Participants in this analysis received their initial ART through ACTG clinical trials and continued their involvement in ACTG research studies for years; therefore, our findings may not be generalizable to all Black individuals with HIV. Participants in this study received different ART regimens, which for many included TDF that can have more pronounced detrimental effects on kidney function than others. Although the effects of *APOL1* variants on kidney function persisted when adjusting for baseline TDF exposure, it is possible that differences in ART regimens may have contributed to greater variability in these renal effects. One limitation of this study was the low occurrence of CVD endpoints; this, along with the relatively young age of the cohort, could explain why we did not identify a relationship between *APOL1* risk alleles and CVD events. Few participants received statins before or during the study, therefore the lack of CVD events could not be attributed to the known beneficial effects of statins in persons with HIV.[44] This study primarily examined individuals through their third to fifth decade of life, and continued future follow-up through the next decade of life may be more informative regarding CVD outcomes. However, the clear effect of HIV viral suppression on preservation of kidney function underscores the value of early interventions.

CONCLUSION

Our findings in this long-term follow-up study confirm the role of *APOL1* genotype in CKD development and the benefits of sustained viral suppression to preserve kidney function and prevent progression to CKD and end-stage kidney disease. However, we did not observe an effect of *APOL1* genotype on CVD, and although hypertension was common in our cohort, we did not observe an effect of *APOL1* genotype on hypertension. In addition, we identified a detrimental effect of 1 *APOL1* risk allele on kidney function, which was less severe than 2 risk alleles and only became apparent in the second decade of follow-up. This study provides important information characterizing long-term health risks among aging people with HIV in a racial group disproportionately impacted by the HIV/AIDS pandemic.

DISCLOSURE

LAB has received royalties from Sanofi-Genzyme for APOL1 research tools that are unrelated to the scope of this manuscript. FJP has received honoraria or consulting fees from EMD Serono, Gilead Sciences, Janssen, ViiV, and Merck. RCK has received research funding from Gilead Sciences. ETO accepted a position at ViiV Healthcare Medical Affairs after the analysis was initiated. All the other authors declared no conflicting interests.

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AUTHOR CONTRIBUTIONS

RCK, LAB, KKT, ETO, FJP, CW, and KW worked on study concept and design. RCK and LAB worked on the study funding and approval. KKT, KW, and RCK worked on data acquisition - statistical analyses; LAB and ZW worked on data acquisition – genotyping. LAB, KKT, and KW drafted the manuscript and prepared figures and tables. RCK, LAB, KKT, ETO, FJP, CW, KW, and ZW edited the manuscript and approved the final version.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1. Association between *APOL1* genotype and incident disease with death treated as a competing risk.

Table S1. Confounding variables examined.

Table S2. Years of follow-up time among all participants.

Table S3. *APOL1* genotype frequencies.

Table S4. Association between *APOL1* genotype and prevalent CKD, CVD and hypertension.

Table S5. Characteristics of the incident hypertension (HTN) group.

Table S6. Characteristics of the incident cardiovascular disease (CVD) group.

Table S7. Characteristics of the incident chronic kidney disease (CKD) group.

Table S8. Age at ART-initiation on study entry by number of *APOL1* risk alleles.

STROBE Checklist.

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