Host APOL1 genotype is independently associated with proteinuria in HIV infection

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Abstract

Proteinuria is associated with adverse clinical outcomes in HIV infection. Here we evaluated whether APOL1 risk alleles, previously associated with advanced kidney disease, is independently associated with proteinuria in a cross-sectional study of HIV-infected women in the Women’s Interagency HIV Study. We estimated the percent difference in urine protein excretion and odds of proteinuria (200 mg/g and higher) associated with two versus one or no
APOL1 risk allele using linear and logistic regression, respectively. Of 1285 women successfully genotyped, 379 carried one and 80 carried two risk alleles. Proteinuria was present in 124 women; 78 of whom had proteinuria confirmed on a second sample. In women without prior AIDS, two risk alleles were independently associated with a 69% higher urine protein excretion (95% CI: 36%, 108%) and 5-fold higher odds of proteinuria (95% CI: 2.45, 10.37) versus one or no risk allele. No association was found in women with prior AIDS. Analyses in which women with impaired kidney function were excluded and proteinuria was confirmed by a second urine sample yielded similar estimates. Thus, APOL1 risk alleles are associated with significant proteinuria in HIV-infected persons without prior clinical AIDS, independent of clinical factors traditionally associated with proteinuria. Trials are needed to determine whether APOL1 genotyping identifies individuals who could benefit from earlier intervention to prevent overt renal disease.

Introduction

Treated HIV infection has evolved into a chronic condition accompanied by multi-morbidities.\(^1\) Proteinuria is particularly common among HIV-infected individuals due to a combination of risk factors, such as hepatitis C virus co-infection (HCV), diabetes, hypertension and HIV viremia.\(^2\) Despite earlier initiation of highly active antiretroviral therapy (HAART), proteinuria remains a strong predictor of worse outcomes in HIV infection, including cardiovascular disease and kidney disease progression.\(^3,4\)

African Americans have long been recognized as having increased risk for end-stage renal disease (ESRD).\(^5\) According to the U.S. administrative data, African Americans are 3.5-times more likely to develop ESRD compared to Whites.\(^5\) Furthermore, the incidence of ESRD attributed to AIDS nephropathy remains 7-fold higher in HIV-infected African Americans compared with Whites.\(^6\)

Animal models and human studies indicate that host genetic background is important in determining the onset and type of kidney disease in HIV-uninfected and infected individuals of African descent. Transgenic mouse models of HIV-associated nephropathy (HIVAN), a disease primarily in individuals of African ancestry, demonstrate that classic histopathological changes require HIV infection of renal epithelial cells and appropriate host genetic background.\(^7\) Two independent genome-wide admixture mapping studies among African Americans with ESRD identified the chromosome 22q region as harboring genes that account for a significant proportion of excess kidney disease risk in African Americans.\(^8,9\) Subsequent studies implicated two APOL1 alleles as culprits.\(^10,11\) The G1 allele is comprised of the two single nucleotide polymorphisms (SNPs) rs73885319 and rs60910145 which are in perfect linkage disequilibrium while the G2 allele consists of rs71785313, a 6-base pair deletion.\(^10\) These variants encode for changes in apolipoprotein L1 that confer resistance against Trypanosoma brucei rhodesiense, the cause of African sleeping sickness, which presumably led to enrichment of these variants among populations of African descent.

Genetic studies thus far suggest a recessive mode of inheritance, with individuals carrying two risk alleles (either G1 or G2) at risk for kidney disease while those carrying one allele having similar risk as those with no risk allele.\(^12,13\) This risk appears augmented in HIV-
infected African Americans. Among these individuals, those with two APOL1 risk alleles had 50% greater risk for HIVAN compared to those with only one or no risk allele. Among those with non-HIVAN renal disease, persons with two APOL1 risk alleles were more likely to have underlying focal segmental glomerulosclerosis (FSGS) rather than immune-complex glomerulonephritis. Moreover, those with two APOL1 risk alleles had a three-fold higher risk of progression to ESRD.

Proteinuria is the primary clinical manifestation of FSGS and HIVAN but can be detected at an earlier stage of kidney disease when it may be more amenable to treatment. The association of APOL1 risk alleles with proteinuria has not been evaluated in the context of HIV infection. Furthermore, whether HIV-related factors such as HIV disease stage or HAART modify the association between APOL1 risk alleles and kidney disease has not been evaluated. Therefore, we conducted a cross-sectional study nested within the Women’s Interagency HIV Study (WIHS) to evaluate the association between APOL1 genotype and proteinuria.

Results

Study Population

Of 1498 HIV-infected women who underwent genotyping for the APOL1 SNPs rs73885319 (G1 allele) and rs71785313 (G2 allele), 154 lacked genetic ancestry data. An additional 59 women failed quality control for APOL1 genotyping. Compared with women included in the study, a greater proportion of excluded women had hypertension (35% vs. 43%, respectively; p=0.02), and fewer were receiving HAART (26% vs. 20%, respectively; p=0.03). Conversely, they had similar sociodemographic characteristics and prevalence for diabetes, HCV co-infection, clinical AIDS history and proteinuria and had similar eGFRs.

Of the 1285 women analyzed, proteinuria was present in 124 women on the first urine sample, 78 of whom had persistent proteinuria on the second sample. A larger proportion of women with prior clinical AIDS (14%) had proteinuria on the first urine sample compared to women without prior AIDS (8%, P=0.03). Eighty (6%) women carried two APOL1 risk alleles while 379 (30%) carried one risk allele. The majority carrying one or two APOL1 risk alleles were African American (Table 1). Women with two APOL1 risk alleles were younger and had a lower prevalence of hypertension compared to women with only one risk allele. Women with two APOL1 risk alleles were also less likely to have received HAART than non-carriers but slightly more likely than women having only one risk allele. Among black women, 79 (10%) carried two APOL1 risk alleles while 343 (43%) carried one risk allele. Among black women who carried two APOL1 risk alleles, 20% had proteinuria on the first sample, and 19% had persistent proteinuria.

Mean Percent Difference in Urine Protein Excretion Associated with APOL1 Genotype

In unadjusted analysis, urine protein excretion from the first sample among women with two APOL1 risk alleles compared to those with one or no risk allele was 40% higher (95% CI: 15%, 72%) (Table 2). In adjusted models, history of clinical AIDS modified the association between APOL1 and urine protein excretion (p-interaction=0.02). Among women without...
prior clinical AIDS, the urine protein excretion in individuals with two APOL1 risk alleles was 69% higher than those with one or no risk alleles (95% CI: 36%, 108%). In contrast, two APOL1 risk alleles were not associated with greater urine protein excretion among women with prior clinical AIDS (beta = −7%; 95% CI: −43%, 50%). HAART use or HCV co-infection did not modify the association between APOL1 and urine protein excretion.

Odds of Proteinuria Associated with APOL1 Genotype

In unadjusted analyses, women with two APOL1 risk alleles had a 3-fold higher odds of proteinuria (95% CI: 1.88, 5.67) (Table 3). In adjusted analyses, clinical AIDS modified the association between APOL1 and proteinuria (p-interaction=0.01). Among women without prior clinical AIDS, those with two risk alleles had a 5-fold increased odds of proteinuria (95% CI: 2.45, 10.37). Among women who had prior clinical AIDS, APOL1 was not associated with proteinuria. In contrast, hypertension, higher HIV-1 viral load and lower kidney function remained independently associated with greater odds of proteinuria in these women.

Sensitivity Analyses

Separate parallel analyses which excluded the 52 women with eGFRs <60 mL/min|1.73 m² and included only black women yielded similar estimates. Among women without prior clinical AIDS and with eGFRs ≥60 mL/min|1.73 m², those with two risk alleles had 62% higher urine protein excretion (95% CI: 32%, 99%) and 4.37-fold greater odds of proteinuria (95% CI: 2.03, 9.41) versus those with one or no risk allele. Similarly among black women, those carrying two risk alleles had 60% higher urine protein excretion (95% CI: 26%, 102%) and 1.60 greater odds of proteinuria (95% CI: 1.26, 2.00). When stratified by AIDS status, black women without prior clinical AIDS carrying two risk alleles had 67% higher urine protein excretion (95% CI: 33%, 109%) and 5.11 higher odds of proteinuria (95% CI: 2.44, 10.68) compared to those carrying either one or no risk allele. In evaluating persistent proteinuria, two APOL1 risk alleles remained significantly associated with greater odds of proteinuria among women without a history of clinical AIDS (OR=5.92; 95% CI: 2.57, 13.67) (Table 4). Among women with prior clinical AIDS, however, there was no association between APOL1 genotype and persistent proteinuria.

Discussion

In this cohort of well-characterized HIV-infected women, APOL1 risk alleles were significantly associated with urine protein excretion and proteinuria in those without prior clinical AIDS, independent of clinical factors traditionally associated with proteinuria in HIV infection. Having two risk alleles was associated with 69% higher urine protein excretion and over fourfold greater odds of proteinuria compared to one or no risk allele. These associations remained significant in sensitivity analyses excluding women with impaired kidney function, excluding women of non-black race, and evaluating proteinuria confirmed on two sequential urine samples. This study is the first to demonstrate that individuals carrying two of either G1 and/or G2 APOL1 risk variants are at higher odds of having early stage kidney disease among HIV-infected individuals.
Proteinuria affects up to one-third of HIV-infected individuals, including those receiving HAART.\textsuperscript{16} It has been associated with significantly elevated risk for mortality\textsuperscript{17} and is an important predictor of renal disease progression in HIV infection.\textsuperscript{4} While HIV-infected individuals often have several risk factors for proteinuria, studies suggest that individuals of African descent are at higher risk for proteinuria in addition to ESRD compared with Whites.\textsuperscript{18,19} Since the initial studies implicating the involvement of $APOL1$ G1 and G2 risk variants in non-diabetic kidney disease among African Americans,\textsuperscript{10,11} subsequent studies confirming this have largely focused on specific renal disease phenotypes and overt kidney dysfunction. Kopp and colleagues reported an odds ratio of 29 for biopsy-proven HIVAN associated with two $APOL1$ risk alleles, although kidney dysfunction severity was not examined.\textsuperscript{14} Among HIV-infected African Americans with biopsy-proven non-HIVAN renal disease and moderate kidney dysfunction (eGFR 41–50 mL/min|1.73 m$^2$), possession of two $APOL1$ risk alleles was associated with a greater propensity for FSGS and hypertensive nephrosclerosis and 3-fold higher risk of progression to ESRD.\textsuperscript{15} The two studies which have evaluated albuminuria in non-diabetic African Americans excluded HIV-infected individuals. In the Dallas Heart Study, African Americans carrying two $APOL1$ risk alleles had a 3-fold higher risk of albuminuria.\textsuperscript{20} Similarly, Freedman and colleagues demonstrated a significant association between $APOL1$ genotype and macroalbuminuria among family members of black individuals with non-diabetic ESRD.\textsuperscript{21} Our findings in HIV-infected persons are consistent with these studies, implicating that $APOL1$ G1 and G2 risk alleles also play an important role in early kidney disease in this patient population. This has public health implications, especially in sub-Saharan African, where an estimated 22.5 million individuals are currently living with HIV and where $APOL1$ risk allele frequencies may reach 45\textpercenter.\textsuperscript{14,22}

Our study did not include HIV-uninfected individuals with which to determine whether HIV status modifies the association between $APOL1$ risk alleles and proteinuria, and comparison to findings in prior studies is difficult due to the differences in the prevalence of non-traditional risk factors for proteinuria among HIV-infected and uninfected individuals such as HCV infection and injection drug use. Previous studies, however, have consistently demonstrated a greater magnitude of association between $APOL1$ risk alleles and HIVAN versus FSGS in HIV-uninfected African Americans. In a case-control study by Papeta and colleagues comparing individuals with HIVAN and HIV-uninfected individuals with FSGS versus healthy controls, the odds of HIVAN associated with $APOL1$ risk alleles in single SNP association analyses ranged from 2.1 to 3.4.\textsuperscript{23} Conversely, the odds of FSGS associated with $APOL1$ risk alleles ranged from 1.8 to 3.0. Kopp and colleagues demonstrated similar trends in a case-control study of HIVAN and non-HIV-related FSGS versus healthy controls in which the odds of HIVAN associated with having two $APOL1$ risk alleles was 29-fold higher while the odds of FSGS was 16-fold higher.\textsuperscript{14} These clinical observations implicate that host gene-viral interactions may be important and are consistent with findings in experimental mouse models. In these models, the genes involved in HIVAN susceptibility appear to modulate expression of genes involved in the podocyte signaling network. The presence of viral proteins augments these alterations to podocyte signaling and lead to overt kidney disease in genetically susceptible mice.\textsuperscript{7}
While our findings support the role of \textit{APOL1} in the development of early kidney disease among HIV-infected individuals, only a minority of women with proteinuria carried two risk alleles suggesting that additional factors are needed in renal disease pathogenesis among HIV-infected persons. Our results are consistent with other studies among African Americans with HIVAN in which 30% and 8% have only one or no risk allele, respectively.\textsuperscript{24} Moreover, the mechanisms by which \textit{APOL1} risk alleles contribute to kidney disease remain unclear. In addition to host-viral interactions, another potential mechanism by which \textit{APOL1} risk alleles promote renal disease development may involve abnormal renal uptake of circulating apolipoprotein L1 or intrinsic renal generation of apolipoprotein L1.\textsuperscript{25} Recent observations support the latter mechanism. In kidney transplantation, donor \textit{APOL1} genotype rather than that of the recipient has been associated with renal allograft failure.\textsuperscript{26,27} Within kidney tissues derived from patients with HIVAN and FSGS, apolipoprotein L1 has been shown to be lower in podocytes and proximal renal tubules compared to those from a healthy kidney.\textsuperscript{28} A preliminary study in which cells were forced to over-express apolipoprotein L1 suggests that \textit{APOL1} G1 and G2 variants may augment autophagy and modify apolipoprotein L1 degradation\textsuperscript{29} perhaps ultimately leading to glomerular loss.\textsuperscript{30}

Surprisingly, we did not observe an association between \textit{APOL1} genotype and proteinuria among women with a history of clinical AIDS. While this suggests that \textit{APOL1} risk alleles may not be necessary for the onset of renal disease in advanced stages of HIV infection, this subgroup was small; therefore, our ability to detect weaker associations was limited. In light of the higher prevalence of proteinuria among women with prior clinical AIDS, other factors related to advanced HIV disease such as opportunistic infections and various drug exposures may have obscured the association between \textit{APOL1} risk alleles and proteinuria in this subset of women. Moreover, women with a history of clinical AIDS and proteinuria may not have been captured in our study due to competing risks such as death or ESRD.

Additional limitations of our study include its cross-sectional design, precluding the evaluation of the onset of proteinuria and determination of the longitudinal trajectory of urine protein excretion associated with \textit{APOL1}. Furthermore, our study lacked renal biopsies to confirm the underlying cause of proteinuria; however, this reflects the general care of HIV-infected patients with kidney disease, the majority of whom do not undergo renal biopsies. As our study was limited to women, our findings may not be generalizable to HIV-infected men; however, current evidence suggests no gender differences with regards to the association of \textit{APOL1} with kidney disease. In addition, our study included a large proportion of participants not receiving HAART; therefore, our results may not be applicable to HAART-treated HIV-infected individuals. Our study was restricted to the early HAART era in which individuals were initiated on HAART at lower CD4+ cell counts than what is currently recommended and in which tenofovir use was infrequent, but our findings are clinically relevant as most individuals present for HIV care with CD4+ cell counts below 350 cells/mm\textsuperscript{3}.\textsuperscript{31}

Strengths of this study include the diverse and well-characterized cohort of HIV-infected women, with assessments of many important factors. A notable proportion of the participants had not initiated HAART, allowing examination of other factors related to
proteinuria in HIV infection which may not be possible in contemporary observational cohorts. Finally, proteinuria was rigorously assessed using urine protein-to-creatinine ratios.

In summary, APOL1 genetic variants are significantly associated with proteinuria independent of traditional and HIV-related risk factors. However, the clinical utility of APOL1 genotyping in HIV-infected persons still needs to be demonstrated before incorporating genetic testing in clinical settings. Trials are also needed to determine whether earlier initiation of HAART or use of medications that block the renin-angiotensin system can curtail the development of proteinuria in genetically susceptible individuals.

Methods

Study design and participants

The WIHS is an ongoing multicenter cohort study of HIV-infected women and women at high-risk for acquiring HIV infection. Study sites include Bronx and Brooklyn, NY; Washington D.C.; San Francisco and Los Angeles, CA; and Chicago, IL. The WIHS design and protocol were previously described in detail. Women were enrolled from October 1994 through November 1995 and from October 2001 through September 2002. Standardized interviews and physical exams are conducted, and biospecimens are collected semi-annually. The study is approved by all local institutional review boards; this cross-sectional study was approved by the Johns Hopkins University Institutional Review Board.

To be included in this study, participants consented to genetic studies and met the following inclusion criteria: 1) were HIV-infected; 2) had self-reported ethnicity-race as Hispanic / non-Hispanic White, Hispanic/ non-Hispanic African American, or other races of Hispanic ethnicity; 3) had at least two stored samples of urine available; 4) had previous genotyping for ancestry; and 5) had DNA available. The sub-study population was similar to the overall WIHS HIV-infected cohort with regards to sociodemographic characteristics (data not shown).

Data Collection

Samples were selected among visits between October 1994 and April 2003 when urine was routinely collected. Two consecutive stored urine samples <1.5 years apart were selected (median 355 days, interquartile range [IQR] 329, 372 days). Urine protein and creatinine were measured using the Siemens Advia 1800 system (Siemens Healthcare Diagnostics, Inc., Tarrytown, NY, USA). Urine protein was measured using the pyrogallol red colorimetric method, with coefficients of variation of 3.3% at 17 mg/dL and 1.8% at 65 mg/dL. Creatinine was measured by Jaffe kinetic alkaline picrate, with coefficients of variation of 2.7% at 67 mg/dL and 3.7% at 145 mg/dL. Proteinuria was defined as a urine protein-to-creatinine ratio ≥200 mg/g on at least one occasion; persistent proteinuria was defined as a ratio ≥200 mg/g or greater on two consecutive samples.

Participants were genotyped for the APOL1 SNPs rs73885319 (G1 allele) and rs71785313 (G2 allele) using TaqMan assays (Applied Biosystems, Inc., Foster City, CA, USA) and then categorized as having two versus one or no risk allele. Participants were previously genotyped for 168 ancestry informative markers using the Illumina GoldenGate platform.
(Illumina, San Diego, CA, USA) to distinguish between West African, European, East Asian and Native American populations. Ten genetic ancestry components were estimated by principal component analysis using EIGENSTRAT software. Additional data included demographic and clinical characteristics and laboratory results at the time of the initial urine collection. History of injection drug use was self-reported. Diabetes mellitus was defined by self-reported use of oral hypoglycemic medications or insulin, fasting blood glucose ≥26 mg/dL, or self-reported history of diabetes diagnosis confirmed by either of the former criteria within 2 years. Hypertension was defined by self-reported use of anti-hypertensive medications, systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or self-reported diagnosis of hypertension confirmed by any of the previous criteria within 2 years. History of clinical AIDS was based on self-reported AIDS-defining illnesses in the 1993 Centers for Disease Control and Prevention class C definition of AIDS and did not include the immunological criteria. Participants’ HAART status were guided by the U.S. Department of Health and Human Services/ Kaiser Panel guidelines. Hepatitis C virus co-infection was defined by a positive antibody and detectable hepatitis C RNA. Glomerular filtration rate (eGFR) was estimated using the serum creatinine-based CKD-EPI equation.

Statistical methods

Baseline characteristics were compared by the number of APOL1 risk alleles using Kruskal-Wallis test and analysis of variance for continuous and chi-squared test for categorical variables. Variables with skewed distributions were log-transformed. We used linear regression models to estimate the mean percent difference in the first urine protein excretion measurement by APOL1 genotype. We used logistic regression models to estimate the odds ratio (OR) and 95% confidence intervals (CI) for proteinuria on the first urine visit associated with having two versus one or no APOL1 risk allele. Models were adjusted for population substructure using the first three genetic ancestry principal components. These principal components were selected based on their significance in the univariable model with each of the outcomes of interest. The remaining covariates in the multivariable models were selected based on their biological association with urine protein excretion and proteinuria and included age, injection drug use, hypertension, HCV co-infection, clinical AIDS history, HAART use, CD4 + cell count, HIV-1 RNA level and eGFR. As few women had diabetes, it was not adjusted for in multivariable models. Effect modification of the association between APOL1 genotype and proteinuria by HCV co-infection, HAART exposure and AIDS history was evaluated by inclusion of interaction terms within models and by stratified analyses. To evaluate the robustness of our estimates, separate sensitivity analyses were performed in which individuals with eGFR <60 mL/min|1.73 m² were excluded, women of non-black race were excluded (since these alleles are most prevalent in individuals of African ancestry), and persistent proteinuria was based on two urine samples. All analyses were conducted using Stata MP 11.2 (StataCorp, College Station, Texas, USA).

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Table 1

Study Participant Sociodemographic and Clinical Characteristics by *APOL1* Genotype

| Characteristic                          | No *APOL1* allele (n=826) | One *APOL1* allele (n=379) | Two *APOL1* alleles (n=80) | P-value |
|----------------------------------------|----------------------------|-----------------------------|-----------------------------|---------|
| Mean age, y (SD)                       | 36.1 (8.1)                 | 37.1 (7.5)                  | 35.5 (7.6)                  | 0.05    |
| Ethnicity/Race, n (%)                  |                            |                             |                             |         |
| Hispanic/ Non-Hispanic White           | 271 (33)                   | 12 (3)                      | 1 (1)                       |         |
| Hispanic/ Non-Hispanic African American| 366 (44)                   | 343 (91)                    | 79 (99)                     |         |
| Hispanic Other                         | 189 (23)                   | 24 (6)                      | 0 (0)                       | <0.001  |
| History of injection drug use          | 248 (30)                   | 125 (33)                    | 24 (30)                     | 0.60    |
| Diabetes mellitus, n (%)               | 26 (3)                     | 16 (4)                      | 3 (4)                       | 0.64    |
| Hypertension, n (%)                    | 185 (22)                   | 118 (31)                    | 18 (22)                     | 0.004   |
| Hepatitis C virus co-infection, n (%)  | 207 (25)                   | 117 (31)                    | 24 (30)                     | 0.41    |
| Clinical AIDS history, n (%)           | 216 (26)                   | 110 (29)                    | 19 (24)                     | 0.47    |
| HAART since last visit, n (%)          | 238 (29)                   | 83 (22)                     | 20 (25)                     | 0.04    |
| Median CD4+ cell count, cells/mm³ (IQR)| 383 (226, 579)             | 396 (222, 598)              | 424 (287, 615)              | 0.29    |
| Median HIV-1 RNA level, 1000 copies/mL (IQR) | 5.4 (0.3, 42.0)           | 6.69 (0.5, 42.0)           | 8.2 (1.1, 30.0)             | 0.77    |
| Median serum creatinine, mg/dL (IQR)  | 0.8 (0.7, 0.9)             | 0.9 (0.7, 1.0)              | 0.9 (0.7, 1)                | 0.001   |
| Mean eGFR, mL/min/1.73 m² (SD)         | 97.8 (81.6, 116.9)         | 100.8 (83.0, 118.2)         | 101.3 (83.3, 124.0)         | 0.55    |
| Median urine protein excretion, mg/g (IQR)† | 59 (40, 87)               | 58 (36, 100)                | 70.5 (45, 185)              | 0.02    |
| Proteinuria, n (%)†                    |                            |                             |                             |         |
| <200 mg/g                               | 762 (92)                   | 338 (89)                    | 61 (76)                     |         |
| 200–500 mg/g                            | 41 (5)                     | 26 (7)                      | 14 (17)                     |         |
| >500 mg/g                               | 23 (3)                     | 15 (4)                      | 5 (6)                       | <0.001  |

† At first urine visit

Abbreviations: y, years; SD, standard deviation; HAART, highly active antiretroviral therapy; QR, interquartile range; eGFR, estimated glomerular filtration rate
Table 2

*APOL1* Risk Alleles and Other Characteristics Associated with Urine Protein Excretion in Unadjusted and Adjusted Linear Regression

| Variables                                      | Unadjusted (n=1285) |   | Adjusted† |   |   |
|------------------------------------------------|----------------------|---|------------|---|---|
|                                                | % Difference | 95% CI | P-value | % Difference | 95% CI | P-value | % Difference | 95% CI | P-value |
| 2 vs. 0/1 *APOL1* risk allele                  | 40          | 15, 72 | 0.01    | 69          | 36, 108 | <0.001 | −7          | 43, 50 | 0.75    |
| Age, per 10 years older                        | 21          | 14, 29 | <0.001 | 8           | 0, 16   | 0.04   | 14          | −4, 36 | 0.14    |
| History of injection drug use                  | 32          | 5, 67  | 0.02    | 32          | 1, 76   | 0.06   | −8          | 41, 43 | 0.71    |
| Hypertension                                   | 41          | 26, 57 | <0.001 | 38          | 20, 58  | <0.001 | 20          | −5, 52 | 0.12    |
| Hepatitis C virus co-infection                 | 32          | 18, 47 | <0.001 | 13          | 1, 29   | 0.08   | −9          | −14, 37 | 0.47    |
| History of clinical AIDS                      | 33          | 19, 48 | <0.001 | ---         | ---     | ---     | ---         | ---     | ---     |
| HAART since last visit                         | −9          | −18, 1 | 0.09    | 5           | −8, 21  | 0.44   | −3          | −27, 28 | 0.82    |
| CD4+ cell count, per ln100 cells/mm³          | −16         | −12, −21 | <0.001 | −13         | −7, −20 | <0.001 | −8          | −17, 3 | 0.14    |
| HIV-1 RNA level, per ln1000 copies/ mL higher | 6           | 4, 8   | <0.001 | 3           | 1, 6   | 0.01   | 7           | 2, 12  | 0.004   |
| eGFR, per 10 mL/min/1.73 m² lower              | 5           | 3, 7   | <0.001 | 2           | −1, 4   | 0.17   | 2           | −3, 7  | 0.46    |

* P-interaction=0.02
† Adjusted for PCs 1–3 and all listed covariates

Abbreviations: PC, principal component; HAART, highly active antiretroviral therapy; eGFR, estimated glomerular filtration rate
Table 3

APOL1 Risk Variants and Other Characteristics Associated with Proteinuria in Unadjusted and Adjusted Logistic Regression

| Variables                           | Unadjusted (n=1285) | Adjusted† Without AIDS History* (n=902) | Adjusted† With AIDS History* (n=326) |
|-------------------------------------|---------------------|-----------------------------------------|-------------------------------------|
| 2 vs. 0/1 APOL1 risk allele         | 3.26                | 5.04                                    | 0.64                                |
| Age, per 10 year older              | 1.57                | 1.17                                    | 1.33                                |
| History of injection drug use       | 1.58                | 0.92                                    | 0.66                                |
| Hypertension                        | 2.22                | 1.87                                    | 2.18                                |
| Hepatitis C virus co-infection      | 1.64                | 1.20                                    | 0.66                                |
| History of clinical AIDS            | 1.77                | 1.20                                    | 0.66                                |
| HAART since last visit              | 0.57                | 0.76                                    | 0.91                                |
| CD4+ cell count, per ln100 cells/mm³ higher | 0.67                | 0.71                                    | 0.62                                |
| HIV-1 RNA level, per ln1000 copies/mL higher | 1.18                | 1.07                                    | 1.25                                |
| eGFR, per 10 mL/min/1.73 m² lower   | 1.18                | 1.12                                    | 1.18                                |

* P-interaction=0.01
† Adjusted for PCs 1–3 and all listed covariates

Abbreviations: PC, principal component; HAART, highly active antiretroviral therapy; eGFR, estimated glomerular filtration rate
### Table 4

**APOL1** Risk Variants and Other Characteristics Associated with Persistent Proteinuria in Unadjusted and Adjusted Logistic Regression

| Variables                                      | Unadjusted (n=1285) | Adjusted† Without AIDS History* (n=902) | Adjusted† With AIDS History* (n=304) |
|------------------------------------------------|---------------------|-----------------------------------------|-------------------------------------|
|                                                | OR                  | 95% CI                                 | P-value                             | OR                  | 95% CI | P-value | OR                  | 95% CI | P-value |
| 2 vs. 0/1 APOL1 risk allele                    | 4.18                | 2.26, 7.74                             | <0.001                              | 1.11                | 0.21, 6.02 | 0.90 |
| Age, per 10 year older                        | 1.59                | 1.21, 2.09                             | 0.001                               | 1.11                | 0.70, 1.75 | 0.65   | 1.23               | 0.64, 2.35 | 0.54 |
| History of injection drug use                  | 0.86                | 0.26, 2.83                             | 0.81                                | 1.16                | 0.24, 5.65 | 0.85   | 1.11               | 0.21, 6.02 | 0.90 |
| Hypertension                                   | 2.48                | 1.55, 3.95                             | <0.001                              | 2.17                | 1.04, 4.51 | 0.04   | 1.93               | 0.79, 4.74 | 0.15 |
| Hepatitis C virus co-infection                 | 1.75                | 1.09, 2.80                             | 0.02                                | 1.18                | 0.56, 2.48 | 0.65   | 1.08               | 0.44, 2.64 | 0.86 |
| History of clinical AIDS                       | 1.77                | 1.10, 2.84                             | 0.02                                | ---                 | --- | --- | --- | --- | --- |
| HAART since last visit                         | 0.82                | 0.48, 1.41                             | 0.48                                | 1.26                | 0.54, 2.94 | 0.58   | 1.20               | 0.37, 3.84 | 0.76 |
| CD4+ cell count, per ln100 cells/mm³ higher   | 0.70                | 0.57, 0.85                             | <0.001                              | 0.69                | 0.47, 1.00 | 0.05   | 0.87               | 0.61, 1.23 | 0.43 |
| HIV-1 RNA level, per ln1000 copies/mL higher  | 1.14                | 1.05, 1.24                             | 0.003                               | 1.06                | 0.91, 1.23 | 0.47   | 1.23               | 1.01, 1.51 | 0.04 |
| eGFR, per 10 mL/min/1.73 m² lower              | 1.20                | 1.10, 1.32                             | <0.001                              | 1.16                | 1.02, 1.32 | 0.03   | 1.14               | 0.94, 1.38 | 0.17 |

*Abbreviations: PC, principal components; HAART, highly active antiretroviral therapy; eGFR, estimated glomerular filtration rate

*P-interaction=0.06

† Adjusted for PCs 1–3 and all listed covariates

‡ History of injection drug use among women with a history of clinical AIDS perfectly correlated with the presence of persistent proteinuria