APOL1 variant alleles associate with reduced risk for opportunistic infections in HIV infection

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Apolipoprotein L1 (APOL1), an innate immune factor against African trypanosoma brucei, inhibits HIV-1 in vitro. The impact of APOL1 G1-G2 variants on HIV-1-associated opportunistic infections (OIs) is unknown. Here, we report findings from a metaanalysis of four HIV/AIDS prospective cohorts (ALIVE, LSOCA, MACS, and WIHS) including 2066 African American participants. Using a global test combining all four cohorts, carriage of two APOL1 variant alleles is associated with a 50% reduction in odds of OI (combined OR 0.50, 95% CI 0.33-0.76). Subgroup analysis of OI etiological categories (viral, parasitic, fungal and Mycobacterial) suggests the possibility of specific protection from fungal infections (OR 0.54, 95% CI 0.32-0.93; \( p_{\text{Bonferroni corrected}} = 0.08 \)). We observe an association of APOL1 variant alleles with host protection against OI in HIV-positive individuals. The study suggests a broader role of APOL1 variant alleles in innate immunity in vivo.

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polipoprotein L1 (APOL1) is a human innate immune factor that is active against African trypanosomes responsible for African trypanosomiasis (sleeping sickness)\(^1\). Two common APOL1 coding alleles, termed G1 and G2, are strongly associated with chronic kidney disease in African-ancestry populations. African Americans carrying two copies of APOL1 G1 or G2 kidney risk alleles (referred to herein as APOL1 variant alleles) have a sevenfold increased risk for nondiabetic end-stage kidney disease and a 17-fold increased risk for focal segmental glomerulosclerosis, respectively\(^2,4\). The strongest associations have been reported for HIV-associated nephropathy, with odds ratio (OR) 29 in African Americans and OR 89 in South Africans\(^3,5\), suggesting a strong interaction between APOL1 and HIV.

APOL1 is a trypanolytic protein that confers innate resistance to African Trypanosoma brucei\(^6\). Individuals with the G2 allele are resistant to acute T.b. rhodesiense Human African trypanosomiasis (HAT) but experience faster progression of chronic T.b. gambiense HAT\(^6\). G1 is associated with asymptomatic carriage and undetectable parasitemia in individuals with chronic T.b. gambiense infection\(^6\). Both forms of HAT may have led to the selection of the APOL1 renal risk alleles in Africa\(^2\). APOL1 also provides resistance against Leishmania\(^7\). It has recently been reported that APOL1 restricts HIV infection in macrophages and differentiated monocytes in vitro\(^8\). The proposed mechanisms include degradation of HIV Gag protein and depletion of HIV Vir, but the in vivo consequences are largely unknown\(^9\).

In addition to its role in innate immunity against trypanosomes, overexpression of the APOL1 variant in monocytes induces differentiation into macrophages, an important component of innate immunity\(^9\). We thus hypothesized that APOL1 could potentially confer resistance to a broad spectrum of pathogens. APOL1 risk variants are found only on African chromosomes and are not present in European or Asian populations, except by African admixture (e.g., African Americans and Afro-Caribbeans). G1- and G2-combined allele frequencies are \(\sim 34\%\) in African Americans and \(10–50\%\) in Sub-Saharan African populations\(^4\), where HIV infection is highly prevalent. Opportunistic infection (OI), caused by a variety of pathogens (bacteria, viruses, fungi, or protozoa), frequently occurs in patients with HIV infection due to a weakened immune system.

In this study, we explored a possible influence of APOL1 variants on opportunistic infections in African Americans from four HIV/AIDS cohorts. We observed that carriage of two APOL1 variant alleles was associated with a reduced risk of OI occurrence. Subgroup analysis of OI etiological categories (viral, parasitic, fungal, and mycobacterial) revealed a tendency of specific protection against fungal infection. Our results suggest that APOL1 variant alleles may confer host protection against OI in HIV-positive individuals.

**Results**

**Baseline characteristics in the MACS, WHS, and LSOCA cohorts.** The genotype distribution of APOL1 genotypes was in concordance with Hardy–Weinberg equilibrium expectations in each of four cohorts \((P > 0.05)\). The HIV-related characteristics of HIV-seroprevalent African Americans at study entry in a part of the ALIVE cohort, the LSOCA, MACS, and WHS cohorts, stratified by APOL1 genotype status, are presented in Table 1. APOL1 variant alleles were present in 8.4, 8.3, 14.4, and 10.5% of HIV-positive participants in these cohorts, respectively.

Baseline HIV viral loads were not statistically different between the APOL1 high-risk and low-risk groups in the ALIVE, LSOCA, MACS, and WHS cohorts \((P = 0.18, 0.49, 0.97, and 0.82, \text{ respectively, Table } 1)\). Baseline CD4 T-cell counts were also not statistically different between the APOL1 high-risk and low-risk groups in the serorepresentative subgroup of the ALIVE cohort, and the serorepresentative LSOCA, MACS, and WIHS cohorts \((P = 0.15, 0.98, 0.68, \text{ and } 0.24, \text{ respectively, Table } 1)\).

**Impact of APOL1 variant alleles on opportunistic infections (OIs).** We evaluated whether APOL1 variant alleles might be protective against opportunistic infections. In ALIVE \((n = 440)\), carriage of two APOL1 variant alleles \((3.9\% \text{ in OI+ vs. } 12.1\% \text{ in OI–})\) was recessively associated with a decreased risk of OIs \((OR = 0.29, P = 0.040; OR_{adj} = 0.32, P_{adj} = 0.044, \text{ Table } 1)\).

We next validated this finding in three other independent HIV-serorepresentative cohorts (LSOCA, MACS, and WIHS) by conducting a meta-analysis of the APOL1 variant allele’s effect on opportunistic infections. In the validation cohorts, carriage of two APOL1 variant alleles \((8.7\% \text{ in OI+ vs. } 14.2\% \text{ in OI–})\) was recessively associated with 36% lower odds of OI (combined OR 0.64, 95% CI 0.45–0.91, \(P = 0.006\), Table 3). We also performed a meta-analysis of APOL1 variant alleles’ effect on OI combining all four HIV cohorts (Table 3). The global test combining the four independent cohorts revealed a significant association of carriage of two APOL1 variant alleles with protection against OI in both unadjusted analysis \((OR 0.56, 95\% \text{ CI } 0.39–0.81, P = 0.002)\) and in an adjusted meta-analysis using covariates (including age, sex, ART usage, and HIV viral load at baseline) \((OR 0.50, 95\% \text{ CI } 0.33–0.76, P = 0.001\), Table 3, Fig. 1). The heterogeneity test revealed no apparent heterogeneity among cohorts \((P_{Q\text{-stats}} ≥ 0.49)\). The leave-one-out meta-analysis removing any one cohort out did not abolish significance (Table 3 and Supplementary Table 1).

Among covariates tested (age, sex, ART treatment history, Pneumocystis pneumonia prophylaxis usage, HIV viral load, and African ancestry), HIV viral load was a consistent significant risk predictor of OI in all cohorts \((OR 1.45, 95\% \text{ CI } 1.12–1.89)\), while ART usage was a protective predictor of OI in two cohorts though short of significance in the meta-analysis of four cohorts \((OR 0.50, 95\% \text{ CI } 0.14–1.72, \text{ Table 3})\). These results are largely consistent with the expectation, attesting to the validity of our model testing.

**Impact of APOL1 variant alleles on fungal infections.** To determine if the APOL1 association was due to a specific OI etiology, we used the LSOCA cohort, which enrolled only participants with an AIDS diagnosis. Dichotomizing specific OI etiologies from the LSOCA cohort revealed that APOL1 variant alleles were associated with a lower risk of fungal OI \((OR = 0.54, P = 0.02)\), and not with viral, parasitic, or bacterial OIs (Table 4).

With a Bonferroni correction of four categories of OIs tested, a trend of fungal protection remained \((P_{\text{Bonferroni corrected}} = 0.08)\) for this exploratory analysis.

Next, to know which individual pathogens are influenced by the APOL1 variants, we performed an explanatory analysis of the top seven most common OIs \((N ≥ 40)\) in the LSOCA cohort. We saw consistent APOL1 variant protective effect trends on fungal OIs, no effect on viral OIs, and possibly increased risk for bacterial pneumonia \((OR = 2.54, P = 0.03)\) (Supplementary Table 2). There is some evidence that variant APOL1 confers partial protection against multiple fungal pathogens, with nonsignificant protective trends for Pneumocystis carinii pneumonia and esophageal candidiasis and a significant protective association with Cryptococcal meningitis \((P = 0.03)\). None of the 47 patients with two APOL1 variant alleles had Cryptococcal meningitis infections. However, we note that these associations did not reach the Bonferroni-corrected significance threshold \((0.05/7 = 0.007)\).
Last, we assessed the association of APOL1 on multiple OI infections (or co-infections). Patients had an average of 2.16 OI diagnoses, most frequently among Pneumocystis carinii pneumonia, esophageal candidiasis, herpes simplex, mycobacterial, and cryptococcal infections (Supplementary Table 3). Using a multivariate regression model, we found that carriage of two copies of APOL1 G1–G2 alleles significantly reduced the number of multiple infections (1.59 vs. 2.17 multiple infections, beta = −0.58, P = 0.03, adjusted for HAART, age, sex, HIV transmission route, and HIV load, Supplementary Table 3).

In the MACS cohort, there was a nonsignificant trend of protection from fungal OI (OR 0.37, 95% CI 0.12–1.14, P = 0.084) and PCP (OR 0.28, 95% CI 0.06–1.28, P = 0.10). The other cohorts, which were smaller, did not have enough subgroup outcomes for meaningful analyses.

Discussion
APOL1 variants have a profound impact on African-ancestry populations in predisposing to a spectrum of progressive kidney diseases, most markedly in those with untreated or undertreated HIV infections\textsuperscript{3,4}. In this study, we assessed the influence of APOL1 variants on susceptibility to opportunistic infections in African Americans from four HIV/AIDS cohorts. Our population genetic epidemiological data revealed a potential role of APOL1 variant alleles in protection against AIDS-related opportunistic infections.

A subgroup analysis of the LSOCA cohort revealed that APOL1 variant alleles are specifically associated with protection against fungal OIs, but not with viral, parasitic, or bacterial OIs. From studying the longitudinal seroconverter ALIVE cohort, we recently reported that APOL1 variants confer no obvious effect on HIV viral load\textsuperscript{11}. This is further confirmed by the results from the seroprevalent patients in the four cohorts included in this study. This suggests that the APOL1 association with OI is unlikely to be mediated by affecting HIV replication but rather, more likely, by inhibiting OI-inducing pathogens directly or via the immune response. The direct inhibition of fungi by overexpression of APOL1 and its variants has recently been demonstrated in vitro\textsuperscript{12}. Expression of human APOL1 reduces yeast S. cerevisiae growth, through impairment of endosomal trafficking and acidification processes\textsuperscript{12}. APOL1 G1 and G2 variant proteins conferred significantly greater toxicity to yeast compared with the wild-type APOL1 G0, likely due to differential impairment of vacuole acidification\textsuperscript{12}. The APOL1 G1 and G2 variant proteins kill T.b. rhodensis by evading virulence factor serum resistance-associated protein (SRA) encoded by the T.b. rhodensiense\textsuperscript{1,10,13,14}. Perhaps, fungi contain a similar counteractive mechanism that differentially interacts with variant APOL1 and APOL1 proteins.

Table 1 Baseline characteristics of seroprevalent participants with HIV infection at study entry by APOL1 variant allele counts in the ALIVE, WIHS, MACS, and LSOCA cohorts.

| Cohort | APOL1 variant allele | Sample size | Male sex, % | Age (SD) | ART use, % | CD4 T-cell counts (SD) | HIV load, log₁₀ (SD) |
|--------|----------------------|-------------|-------------|-----------|------------|------------------------|---------------------|
| ALIVE\textsuperscript{a} | 1 or 0 | 196 (91.6%) | 74.5 | 41.1 (6.1) | 25.7 | 558.5 (278.9) | 4.0 (0.92) |
| | 2 | 18 (8.4%) | 83.3 | 43.4 (3.1) | 23.1 | 669.2 (296.0) | 3.66 (0.97) |
| | P value | 0.41 | 0.12 | 0.73 | 0.15 | 0.18 | 0.18 |
| LSOCA | 1 or 0 | 719 (91.7%) | 67 | 42.6 (9.0) | 82 | 227 (212) | 3.3 (1.4) |
| | 2 | 65 (8.3%) | 71 | 43.9 (7.4) | 94 | 227 (215) | 3.2 (1.4) |
| | P value | 0.52 | 0.5 | 0.02 | 0.98 | 0.49 | 0.49 |
| MACS | 1 or 0 | 557 (85.6%) | 100 | 35.5 (8.4) | 72.4 | 573.7 (359.0) | 2.99 (1.29) |
| | 2 | 94 (14.4%) | 100 | 34.3 (8.3) | 72.3 | 485.2 (316.2) | 2.98 (1.31) |
| | P value | 1.0 | 0.22 | 1.0 | 0.68 | 0.97 | 0.97 |
| WIHS | 1 or 0 | 913 (89.5%) | 0 | 36.3 (8.0) | 16.5 | 448.9 (297.3) | 3.78 (1.14) |
| | 2 | 107 (10.5%) | 0 | 35.2 (7.4) | 15 | 485.2 (316.2) | 3.80 (1.05) |
| | P value | 1.0 | 0.16 | 0.67 | 0.24 | 0.82 | 0.82 |

\(SD\) standard deviation, ART antiretroviral therapy.
\(P\) values were from a Chi-squared test for categorical comparisons and a \(t\) test for continuous variable comparisons.
\(a\)Fisher’s exact test.

Table 2 Association of APOL1 G1 and G2 variant alleles with opportunistic infections in HIV-positive individuals in the ALIVE cohort.

| APOL1 variant allele | OI \(-, n = 363\) (%) | OI \(+, n = 77\) (%) | OR (95% CI) | \(P\) |
|----------------------|-----------------------|----------------------|-------------|-------|
| 0 | 142 (39.1) | 35 (45.5) | Ref. | 1 |
| 1 | 177 (48.8) | 39 (50.7) | 0.89 (0.54–1.48) | 0.67 |
| 2 | 44 (12.1) | 3 (3.9) | 0.28 (0.08–0.94) | 0.034\textsuperscript{a} |
| Additive (2 vs. 1 or 0) | 0.29 (0.08–0.97) | 0.040\textsuperscript{b} |
| 2 vs. 1 or 0 | 0.29 (0.09–0.98) | 0.044\textsuperscript{b} |

\(a\)Adjusting for proportions of African ancestry using the first five principal components (PC), HIV-1 viral load, and age in logistic regression.

Showed are the rates of opportunistic infection (OI) among subjects with carriage of 2 and 1 or 0 APOL1 variant alleles. HIV-positive individuals included both seroconverters (\(n = 226\))\textsuperscript{11} and seroprevalent (\(n = 214\), see Table 1). The additive model approach statistical significance and the recessive model reached statistical significance.
Variant APOL1 may also affect susceptibility to OI through immune activation and enhancement. Host susceptibility to pathogen invasion is strongly determined by the robustness of the innate immune response, as adaptive immune response takes days to develop. APOL1 is upregulated by pro-inflammatory cytokines such as IFN-γ, IFN-β, IFN-α, and TNF, which are induced by invading viruses and other pathogens, including HIV and fungi. APOL1 and APOL1 variant protein may differentially induce macrophage polarization and modify immune responses. APOL1, under stimulation of pro-inflammatory cytokines IFNγ and lipopolysaccharide, a major component of the outer membrane of Gram-negative bacteria, induced differentiation of THP-1 monocytic cells into the polarization of typical M1 macrophage state. APOL1 G1 and G2 variants induced more IL6 and TNF mRNA (M1 marker), a presumably stronger M1 state, compared with APOL1-G0 protein that carried no variant alleles. Macrophages are among the first-line effectors of the innate immune defense against invading pathogens and chemokines to recruit other immune cells to control the infection. Together, these data support differential roles of APOL1 protein isoforms in the immune defense of OI, although the mechanisms remain unresolved.

APOL1 expression is increased by elevated circulating levels of interferon in several clinical settings, leading to glomerular injury. These settings include (1) chronic viral infection, e.g., with HIV infection (as discussed above) and with parvovirus B19 infection, (2) administration of therapeutic interferon, given for other indications, and (3) a genetic disorder, e.g., the stimulator of interferon (STING)-associated vasculopathy with onset in infancy (SAVI). It is likely that this list will grow longer.

The studies add to evidence that APOL1 or its variant isoforms may confer protection to a broader range of pathogens than only African trypanosomes. APOL1 has been shown to confer resistance against Trypanosoma brucei, amelioration of Leishmania infection, and inhibition of HIV-1 replication in certain cell types. APOL1 G1 and G2 variants exhibit recent positive selection signals in the form of extended haplotypes in some West Africa populations, possibly due to selective pressure from pathogens during recent evolution. The broader role of APOL1 as an innate immune factor against fungal pathogens, if validated, may explain in part why APOL1 G1 and G2 variants have been selected in African-ancestry populations, despite the increased risk for kidney disease and preeclampsia. A protective impact of APOL1 variant alleles against OIs could therefore influence HIV disease outcomes among African-ancestry populations and have implications for targeted management. It is possible that APOL1 G1 and G2 variants could be under positive selective pressure from OIs, in addition to T.b. rhodesiense and T.b. gambiense. In addition to pleiotropic associations of APOL1 risk alleles with human African trypanosomiasis, kidney disease, cardiovascular disease, and preeclampsia, carriage of APOL1 risk alleles was unexpectedly found to be associated with an elevated risk of sepsis in a study of older, community-dwelling black participants enrolled in the REGARDS (reasons for geographic and racial differences in stroke). This is consistent with our observation of APOL1 risk alleles increasing the risk of bacterial pneumonia, as most sepsis is caused by bacterial infections. APOL1 variants may have pleiotropic-modifying effects on innate immune response or inflammatory responses to different classes of human pathogens.

There are limitations to our study. The strength of statistical association for OI is modest or nonsignificant in individual cohorts, likely due to the relatively low frequency of APOL1 variant alleles and the modest sample size. With the sample size in the combined cohorts, assuming a 30% OI prevalence rate, we had 80% power to
The study should be considered as exploratory and hypothesis-generating. More studies with a larger sample size are required to reach a definitive conclusion and to elucidate specific mechanisms leading to protection against OI. It also remains to be determined if the APOL1 association with OI in the settings of HIV infection extends to other settings of immune suppression (e.g., individuals with nephrotic syndrome or chronic and end-stage kidney disease experiencing uremia, or transplant recipients and cancer patients taking immunosuppressive drugs).

In summary, this population genetic study suggested that APOL1 might confer carriers of two variant alleles’ protection from HIV-related opportunistic infections, especially fungal infections. These findings warrant further replication and experimental validation and extension to infectious disease incidence and prevalence in populations of recent African ancestry, particularly those with chronic kidney disease and end-stage kidney disease and those immunocompromised due to many other diseases.

### Methods

#### Ethics statement
Ethical approval for the study was obtained from the National Institute of Health Office of Human Subjects Research Protections (OHSRP #3314). Institutional Review Boards of all participating institutions approved the study protocols and written informed consent was obtained from all study participants.

#### Study participants
We studied African American subjects enrolled in four US-based HIV cohorts since APOL1 G1–G2 alleles are only present in individuals with recent African ancestry. The four HIV cohorts include the ALIVE, consisting of half seroconverters and half of the seroprevalence, and the seroprevalent cohorts LSOCA, MACS, and WHIS.

**The ALIVE cohort.** The epidemiological and clinical characteristics of the ALIVE (AIDS link to the intravenous experience) cohort have been previously described26. ALIVE is a prospective longitudinal cohort originally designed to characterize the incidence and natural history of HIV infection among injection drug users (IDU) in Baltimore, MD, initiated in 198826. The participants were followed at six-month intervals with blood draws for viral load and CD4+ T-cell measurements and physical exam at each visit. The censoring date used was the date of the last recorded visit, if prior to July 21, 1997, otherwise a date of July 31, 1997, was used, in order to minimize the confounding effect of antiretroviral therapy (ART)27. The study group includes 227 African American incident HIV seroconverters and 213 HIV-seropositive individuals (acquired HIV prior to study entry).

**The LSOCA cohort.** The LSOCA (longitudinal study or the ocular complications of AIDS) was a multicenter prospective observational study of patients diagnosed with AIDS28. The study was originally designed for the occurrence and consequences of ocular opportunistic infections, particularly cytomegalovirus (CMV) retinitis, among patients with AIDS. Participants were enrolled at 19 clinical centers throughout the United States in 1998–2011. Each patient gave a detailed medical and HIV-related disease history and relevant findings were confirmed from the medical records. At least every 6 months, patients were examined and the standardized data were collected on AIDS history and treatment, eye examinations, and hematologic, virologic, and immunologic laboratory data29–31. Only baseline data at study entry were used in this study, as all these seroprevalent participants (n = 784) already had AIDS at the study entry.

### Table 4 Association of APOL1 variant alleles with baseline AIDS-defining opportunistic infections among LSOCA participants under additive and recessive genetic models.

| Outcome                  | Total OI | Additive model | Recessive model (HR) |
|--------------------------|----------|----------------|----------------------|
|                          |          | OR_{adj}       | p_{adj}              | OR_{adj} | p_{adj} |
| Any viral OI\(a\)        | 161      | 0.91 (0.68-1.23) | 0.54                 | 0.98 (0.66-1.39) | 0.95 |
| Any parasitic OI\(b\)    | 27       | 0.86 (0.73-0.98) | 0.20                 | 0.77 (0.44-1.34) | 0.36 |
| Any fungal OI\(c\)       | 412      | 0.78 (0.63-0.98) | 0.03                 | 0.54 (0.32-0.93) | 0.02 |
| Any mycobacterial OI\(d\)| 152      | 1.17 (0.87-1.57) | 0.31                 | 1.06 (0.54-2.10) | 0.87 |
| Any OI\(e\)              | 546      | 0.87 (0.69-1.09) | 0.22                 | 0.66 (0.40-1.11) | 0.11 |

- \(a\) Viral opportunistic infections include any CMV, Kaposi sarcoma-related herpes virus, and herpes simplex virus.
- \(b\) Parasitic opportunistic infections include cerebral toxoplasmosis infections, cryptosporidiosis, isosporiasis, and extrapulmonary pneumocystosis.
- \(c\) Fungal opportunistic infections include Pneumocystis carinii pneumonia (PCP), candidiasis, cryptococcosis, histoplasmosis, and coccidiomycytical infections.
- \(d\) Bacterial opportunistic infections include Mycobacterium tuberculosis, Mycobacterium avium complex (MAC), and other mycobacterial infections.
- \(e\) Subjects with at least one opportunistic infection of any class.
- Adjusted for ART, age, sex, and HIV transmission routes.

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**Fig. 1** Forest plot showing the odds ratios (OR) of opportunistic infection (OI) for the carriage of 2 versus 1 or 0 APOL1 variant alleles in the meta-analysis of four HIV/AIDS cohorts. Data were pooled from the four studies using inverse variance (IV) (Table 3). The red block indicates the point estimate of OR, while the horizontal line depicts 95% CI of OR in each study. The area of the block indicates the weight assigned to that study in the meta-analysis.
The MACS cohort. Multicenter AIDS Cohort Study (MACS) is a longitudinal prospective cohort of men who have sex with men from four US cities: Chicago, Baltimore, Pittsburgh, and Los Angeles, with enrollment starting in 1984. Participants were followed at 6-month intervals. The data-censoring date was the earliest of the date of the last recorded visit, or December 31, 1995. In this study, 651 HIV-positive African Americans (90% seroreconverters) with complete APOL1 genotype, phenotype, and covariate information were included; their enrollment date ranged from 1984 to 2003 with an average follow-up of 10 years. The WHI Cohort. The Women's Interagency HIV Study (WHIS) is the largest multicenter longitudinal cohort of HIV-positive women in the United States, starting in 1994–1995. Participants were seen at 6-month intervals for laboratory and physical examinations. The current analysis included 91 seroconversion HIV-positive non-Hispanic black women with APOL1 genotype and OI diagnostic information at study entry.

Diagnosis of opportunistic infections. OIs were defined as per CDC-revised 1993 AIDS case definition and the MMWR Recommendations. The classification of opportunistic infections was made according to the AIDS Clinical Trials Group guidelines. Different etiologies for OIs were available for the LSOCA cohort, allowing us to test for associations with the following subcategories: viral OI (including CMV, Kaposi sarcoma-related herpes virus, and herpes simplex virus), parasitic OIs (including extrapulmonary pneumocystosis, toxoplasma infections, cryptococcosis, and isosporiasis), fungal OI (including Pneumocystis carinii, candidiasis, cryptococcal, histoplasmosis, and coccidioidomycosis infections), and bacterial OI (including Mycobacterium tuberculosis, Mycobacterium avium complex, Mycobacterium kansasii, Mycobacterium genovensis, and other mycobacterial infections).

Genotyping of APOL1 G1–G2 variant alleles. APOL1 coding variants G1 (rs73885319, p.S342G) and G2 (rs73875313, p.N388_Y389del) were genotyped using ABI TaqMan genotyping assays on an ABI 7900HT sequence detection system (Applied Biosystems, Foster City, CA), as previously described. For quality control, water controls were included on each plate and 10% of samples were duplicated. No water contamination or genotype mismatches between duplicates were observed. G1 and G2 allele calls were also validated in the ALIVE cohort by the Sangamo sequencing, following a previously described protocol. The APOL1 variants were genotyped in the Winker lab for the ALIVE and LSOCA cohorts. The MACS and WHIS participants were genotyped by the cohort studies using Taqman protocols, as previously published.

Defining APOL1 variant alleles. The APOL1 G1 variant allele is defined by the presence of rs73885319 G1 (342G), which is in almost complete positive linkage disequilibrium with rs73885313 (384M), and the G2 variant allele by rs73875313, an in-frame 6-base deletion (TTAATA), leading to the loss of two amino acids (p.N388_Y389del); the G0 haplotype contains neither the G1 nor the G2 variant allele. G1 and G2 variant alleles are in absolute negative disequilibrium and always occur on different chromosomes. Individuals carrying any two variant alleles in the homozygous or compound heterozygous state (G1/G1, G1/G2, G2/G2, or G1/G2) are considered APOL1 high-risk (HR) carriers and are at increased risk for kidney disease; individuals carrying 0 or 1 risk allele are defined as APOL1 low-risk carriers (LR) for kidney disease.

Statistical analysis. We evaluated the effects of APOL1 variant alleles using an additive model, dominant model (2 vs. 1 or 0 copies), recessive model (2 vs. 1 or 0 copies), and a regression model using SAS version 9.12 (SAS Institute, Cary, NC). We tested Hardy–Weinberg equilibrium (HWE) of APOL1 variant genotypes by using a goodness-of-fit χ² test and an exact test.

We compared the mean baseline CD4 T-cell counts between the group carrying two APOL1 variant alleles and the group carrying 1 or 0 variant alleles using ANOVA. We adjusted the regression model analyses by sex and by age at seroconversion, or age at study entry for those who were seroreverters, using the following age categories: 0–19, 20–40, and >40 years.

To account for potential population stratification among participants, we adjusted the regression model association tests in the ALIVE and WHIS cohorts using the first five eigenvalues generated with principal component analysis (PCA) implemented in Eigenstrat, using African-ancestry informative markers or GWAS data; the PCA data were not available for the particular LSOCA and MACS datasets used in this study.

Analysis of opportunistic infections (OIs). We evaluated the impact of APOL1 variant alleles on OI acquisition by comparing the frequencies of APOL1 genotypes between those with OI and those without OI among all HIV-positive subjects in the ALIVE (including seroconverters and serorevertent subjects) using OI outcomes at the cohort- censoring date. For the WHIS and LSOCA cohorts, OI status at study enrollment was used to minimize the influence of ART and prophylaxis on OI outcomes. Odds ratios (OR) and two-tailed P values were obtained by chi-square tests or using a conditional logistic regression model. The regression model was adjusted for age, sex, HIV-1 viral load, ART use, and OI prevention medications, and proportions of African ancestry using the first five principal components (PC), based on the data available from each cohort. We observed the Bonferroni multiple testing-correlated P value with p.adjust function from the stats package in R.

Meta-analysis. Meta-analysis was performed by calculating the inverse variance of OR and 95% CI in a random-effects model as implemented in the RevMan V.5.3 software (Cochrane Community, Copenhagen). Statistical heterogeneity between studies was assessed by calculating tau-squared (τ²), chi-squared (χ²) test, P values, and I². Under a random-effects model in the meta-analysis, the variance of the distribution of true effect sizes was estimated by τ². A low P value provides evidence of variation in effect estimates beyond chance. The I² statistic describes the fraction of variance across studies that is due to heterogeneity.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. The genotype data of HIV seroconverters in the ALIVE cohort were previously reported and can be accessed at doi: 10.3389/fimmu.2019.00053. All other data that support the findings of this study are included in this published article and its Supplementary Information files or are available from the corresponding authors on reasonable request.

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References
1. Pays, E. et al. The trypanolytic factor of human serum. Nat. Rev. Microbiol. 4, 477–486 (2006).
2. Genovese, G. et al. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. Science 329, 841–845 (2010).
3. Kopp, J. B. et al. APOL1 genetic variants in focal segmental glomerulosclerosis and HIV-associated nephropathy. J. Am. Soc. Nephrol. 22, 2129–2137 (2011).
4. Limou, S., Dummer, P. D., Nelson, G. W., Kopp, J. B. & Winkler, C. A. APOL1 toxin, innate immunity, and kidney injury. Kidney Int. 88, 28–34 (2015).
5. Kasembeli, A. N. et al. APOL1 risk variants are strongly associated with HIV-associated nephropathy in Black South Africans. J. Am. Soc. Nephrol. 26, 2882–2890 (2015).
6. Cooper, A. et al. APOL1 renal risk variants have contrasting resistance and susceptibility associations with African trypanosomiasis. eLife 6, e25461 (2017).
7. Samanovic, M., Molina-Portela, M. P., Chessler, A. D., Burleigh, B. A. & Raper, J. Trypanosome lytic factor, an antimicrobial high-density lipoprotein, ameliorates Leishmania infection. PLoS Pathog. 5, e1000276 (2009).
8. Taylor, H. E., Khatua, A. K. & Popik, W. The innate immune factor apolipoprotein L1 restricts HIV-1 infection. J. Virol. 88, 592–603 (2014).
9. Lee, H. et al. Apol1 renal risk variants induce aberrant TH1 monocyte differentiation and increase eicosanoid production via enhanced expression of cyclooxygenase-2. Am. J. Physiol. Ren. Physiol. 315, F140–F150 (2018).
10. Dummer, P. D. et al. APOL1 kidney disease risk variants: an evolving landscape. Semin Nephrol. 35, 222–236 (2015).
11. An, P. et al. Impact of APOL1 genetic variants on HIV-1 infection and disease progression. Front. Immunol. 10, 53 (2019).
12. Kruzel-Davila, E. et al. APOL1-mediated cell injury involves disruption of conserved trafficking processes. J. Am. Soc. Nephrol. 28, 1117–1130 (2017).
13. Thomson, R. et al. Evolution of the primate trypanolytic factor APOL1. Proc. Natl Acad. Sci. USA 111, E2130–E2139 (2014).
14. Smith, E. E. & Malik, H. S. The apolipoprotein L family of programmed cell death and immunity genes rapidly evolved in primates at discrete sites of host-pathogen interactions. Genome Res. 19, 850–858 (2009).
15. Erweg, L. P. & Gom, N. A. Interactions of fungal pathogens with phagocytes. Nat. Rev. Microbiol. 14, 163–176 (2016).
16. Nichols, B. et al. Innate immunity pathways regulate the nephropathia gene Apolipoprotein L1. Kidney Int. 87, 332–342 (2015).
17. Zhao, H., Chen, W., Gao, G., Kini, R., Jiang, Z. & Hu, C. A. ApoL1, a BH3-only lipid-binding protein, induces autophagic cell death. Autophagy 4, 1079–1082 (2008).
18. Mege, J. L., Mehraj, V. & Capo, C. Macrophage polarization and bacterial infections. Curr. Opin. Infect. Dis. 24, 230–234 (2011).
19. Xu, S. & Shinohara, M. L. Tissue-resident macrophages in fungal infections. Front. Immunol. 8, 1798 (2017).
