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Association between *APOL1* risk variants and progression from infection to sepsis

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Abstract

Importance

Two risk variants in the apolipoprotein L1 gene (*APOL1*) have been associated with increased susceptibility to sepsis in Black patients. However, it remains unclear whether *APOL1* high-risk genotypes are associated with either progression from infection to sepsis or sepsis-related phenotypes, independent of their association with severe renal disease.

Objective

To examine the association between *APOL1* high-risk genotypes and the risk of progression from infection to sepsis and sepsis-related phenotypes.

Design, setting, and participants

A retrospective cohort study of 2,242 Black patients hospitalized with an infection.

Exposures

Carriage of *APOL1* high-risk genotypes.

Main outcomes and measures

The primary outcome was sepsis; secondary outcomes were death and organ failure related to sepsis.

Results

Of 2,242 Black patients hospitalized with infections, 565 developed sepsis. Patients with high-risk *APOL1* genotypes had a significantly increased risk of sepsis (odds ratio [OR]=1.29 [95% CI, 1.00–1.67; $p=0.047$]); however, this association was not significant after adjustment for pre-existing severe renal disease (OR=1.14 [95% CI, 0.88–1.48; $p=0.33$]), nor after exclusion of those patients with pre-existing severe renal disease (OR=0.99 [95% CI, 0.70–1.39; $p=0.95$]). *APOL1* high-risk genotypes were significantly associated with the renal dysfunction component of the Sepsis-3 criteria (OR=1.64 [95% CI, 1.21–2.22; $p=0.001$], but not with other sepsis-related organ dysfunction or death. The association between high-risk *APOL1* genotypes and sepsis-related renal dysfunction was markedly attenuated by adjusting for pre-existing severe renal disease (OR=1.36 [95% CI, 1.00–1.86; $p=0.05$]) and was nullified after exclusion of patients with pre-existing severe renal disease (OR=1.16 [95% CI, 0.74–1.81; $p=0.52$]).

Conclusion and relevance

APOL1 high-risk genotypes were associated with an increased risk of sepsis; however, this increased risk was attributable predominantly to pre-existing renal disease.

eLife assessment

In this **valuable** study, patients homozygous for both minor frequency alleles of the *APOL1* gene are shown to be at significant risk for progression into sepsis after infection. The study has enrolled a significant number of subjects and provides **solid** results. The study addresses to infectious diseases and critical care experts and one major weakness is the lack of inclusion of non-Black patients.

Introduction

Sepsis is a common cause of morbidity and mortality in the United States, accounting for one in every two to three deaths that occur in hospitals.¹ The risk of sepsis and associated mortality are approximately 60% and 80% higher, respectively, for Black patients compared to White patients.^{1,2} The higher incidence of sepsis in Black individuals persists after adjustment for comorbidities and socioeconomic status, encompassing both a greater risk for developing infection, and once infected, a greater risk of organ dysfunction.³ Recent work in the Million Veteran Program (MVP) suggests that variants in the apolipoprotein L1

gene (*APOL1*) that are common in people of African ancestry may play a role in the pathogenesis of sepsis.⁴

Two genetic variants in *APOL1*—termed G1 (rs73885319/rs60910145) and G2 (rs71785313)—are found almost exclusively in individuals of African ancestry and confer resistance to *Trypanosoma brucei* infection.^{5–8} However, individuals carrying two such alleles (i.e., G1/G1, G1/G2, or G2/G2) have a marked increase in the risk of chronic renal disease; for example, among African Americans, individuals carrying two *APOL1* risk alleles are 7.3 times more likely to develop hypertension-associated end-stage renal disease (ESRD) compared to those without two risk alleles.⁵ Correspondingly, carriage of two risk alleles is associated with an increased prevalence of numerous renal-related disorders, including hypertension, focal segmental glomerulosclerosis, and HIV-associated nephropathy.^{9–11} While these high risk alleles typically follow a pattern of recessive expression (i.e., without increased risk for individuals carrying a single allele), because their carriage rates are so high among African-Americans (10-15% of African-Americans carry two *APOL1* risk alleles), a substantial portion of this population faces increased *APOL1*-related risk.⁵

The specific mechanisms whereby the *APOL1* risk variants increase the risk for renal disease are not fully understood, but beyond their role in innate immunity and resistance to trypanosomiasis, the *APOL1* risk variants are considered gain-of-injury variants,¹² intensifying autophagy, cell death, endothelial cell inflammation and dysfunction, and immune pathway activation.^{4,13,14} However, many patients with two *APOL1* risk variants never develop renal disease, leading researchers to propose a 2-hit model in which genetic susceptibility combined with an inflammatory trigger leads to disease.¹² Indeed, *APOL1* expression is induced by inflammatory cytokines such as tumor necrosis factor alpha and gamma-interferon,^{15,16} thus increased transcription of the gain-of-function variant *APOL1* is most likely to occur in the setting of severe infection.

Given the substantive risks and costs associated with sepsis, as well as the reported association between sepsis and the presence of two *APOL1* risk alleles,⁴ it is critical to better understand whether the relationship between *APOL1* and sepsis is causal. In particular, several inhibitors of *APOL1* are in various stages of development—if *APOL1* high-risk genotypes are causally related to the development of sepsis, such drugs could be applied to high-risk patients with infection to prevent progression to sepsis.¹⁷

The objective of this study was to better understand the relationship between *APOL1* and sepsis, focusing on two critical points. First, whether *APOL1* high-risk genotypes are associated with the risk of *progression* from infection to sepsis, independent of their association with severe renal disease. Second, among patients carrying *APOL1* high-risk genotypes, whether the risk of organ dysfunction that defines the presence of sepsis is limited to renal dysfunction *or* if it affects other typical organ system dysfunction components of sepsis (i.e., hepatic, respiratory, circulatory, and hematologic dysfunction).

Methods

Study population and design

This study used data from the Vanderbilt University Medical Center (VUMC) Synthetic Derivative, which contains a de-identified version of the electronic medical records (EHR) for VUMC patients (~3.6 million individual records as of October 2022). These de-identified EHRs are linked to a biobank (BioVU), which has genome-wide genotyping results for ~120,000 patients. From these genotyped patients, we constructed a cohort of Black patients admitted to the hospital with an infection (infection cohort) to assess the association

between carriage of *APOL1* high-risk alleles and progression from infection to sepsis before and after consideration of pre-existing renal disease. To examine the findings of the MVP sepsis study⁴ in our cohort, we performed a cross-sectional restricted phenome-wide association analysis (PheWAS) in all Black patients with existing genotypes to define the association between the carriage of *APOL1* high-risk genotypes and phenotypes previously reported to be associated with sepsis.⁴ International Classification of Disease, ninth revision, Clinical Modification (ICD-9-CM); tenth revision (ICD-10-CM); and Current Procedural Terminology (CPT) codes were used for cohort construction and covariates. This study was reviewed by the VUMC Institutional Review Board and determined to be non-human subjects.

Infection cohort

Inclusion/Exclusion Criteria

The infection cohort included individuals with EHR-reported Black race who were admitted to the hospital with an infection between Jan 2000 and Aug 2020 and were ≥ 18 years old on the day of admission.¹⁸ EHR-reported race is highly consistent with genetic ancestry in BioVU.¹⁹ The day of hospital admission was designated day 0. Infection was defined as having a billing code indicating an infection and receiving an antibiotic within one day of hospital admission (i.e., on days -1, 0, or +1).^{18,20,21} We used ICD-9-CM and ICD-10-CM codes for this definition of infection based on the criteria of Angus *et al.*^{22,23} excluding viral, mycobacterial, fungal, and spirochetal infections, as we have described in detail previously.^{18,20,21} Only the first hospitalization for infection was included if a patient had more than one qualifying episode. We excluded individuals admitted for cardiac surgery, cardiogenic shock, and organ transplantation, as well as those with no relevant laboratory values (i.e., creatinine, bilirubin, or platelets) on days -1, 0, or +1. We also excluded patients who had a positive test or ICD-10-CM code (U07.1) for coronavirus disease (COVID-19) on days -1, 0, or +1.^{18,20,21}

Outcomes

The primary outcome was the development of sepsis as indicated by fulfillment of the Sepsis-3 criteria (described below). Secondary outcomes were the individual organ dysfunction criteria in the Sepsis-3 definition (renal, hepatic, respiratory, circulatory, and hematologic dysfunction) as well as severe sepsis/septic shock, and death.

Sepsis was defined by the Sepsis-3 criteria of concurrent infection and organ dysfunction (**Supplementary Figure 1**)^{23,24} using the EHR definition that was developed in real-world hospital settings^{24,25} and optimized, validated, and applied across EHR systems from 409 hospitals.²⁴ The algorithm uses billing codes and clinical criteria, with a specificity of 98.1% and a sensitivity of 69.7%.²⁴ We have adapted and applied the EHR-based Sepsis-3 algorithm to the de-identified EHRs in our system, as described previously.^{20,21} Because the vast majority of community acquired sepsis cases (87%) are present on admission to hospital,²⁴ we studied sepsis occurring within one day of hospital admission (days -1, 0, and +1) to minimize the confounding effects of sepsis occurring secondary to procedures or events in the hospital. We have previously validated this algorithm in our EHRs.^{18,20,21}

In brief, individuals in the infection cohort met the definition of sepsis if they had either ICD codes for septic shock or severe sepsis (ICD-9-CM, 995.92 and 785.52; ICD-10-CM, R65.20 and R65.21) because these are highly specific (99.3%),²⁴ or they met any Sepsis-3 criterion for serious organ dysfunction (**Supplementary Figure 1**).²⁴ Criteria for organ system

dopamine unrelated to stress echocardiography (CPT codes 78452, 93015, 93018, 93016, 93017, and 93351) and with at least two mentions of any of the keywords (i.e., “infection,” “sepsis,” or “septic”); (2) *respiratory*: defined by ICD and CPT codes for ventilation and admission to an ICU; (3) *renal*: defined by a doubling or greater increase of baseline creatinine (baseline creatinine was defined as the lowest creatinine between 1 year before admission and hospital discharge); (4) *hepatic*: defined as a total bilirubin ≥ 34.2 $\mu\text{mol/L}$ (2 mg/dL) and at least double from baseline (baseline bilirubin was defined as the lowest total bilirubin occurring between 1 year before admission and hospital discharge); and (5) *hematologic*: defined as a platelet count $<100,000$ /microL and $\geq 50\%$ decline from a baseline that must have been $\geq 100,000$ (the baseline value was the highest platelet count occurring between 1 year before admission and hospital discharge).^{18,20,21} Deaths were defined as patients who 1) had death recorded in the EHR within the index hospital stay or 2) had been discharged to hospice.²⁶

Covariates

We extracted demographic characteristics from the EHRs, including sex and age at the time of the index hospital admission. Comorbidities were collected^{27,28} using relevant diagnostic codes in the year before the index hospital admission (**Supplementary Table 1**) grouped into the 17 Charlson/Deyo comorbidity categories.^{29–31} We also identified patients with pre-existing severe renal disease (i.e., Stage 4/5 chronic kidney disease/ESRD) as evidenced by one or more of the following ICD diagnosis and procedure codes: N18.4, N18.5, N18.6, N18.9, 585.4, 585.5, 585.6, 585.9, 586, Z99.2, Z49.0, Z49.31, 39.95, V45.11, V56.0, **Supplementary Table 2**). Principal components (PCs) for ancestry were calculated using common variants (minor allele frequency [MAF] $>1\%$) with a high variant call rate ($>98\%$), excluding variants in linkage and regions known to affect PCs (i.e., the HLA region on chromosome 6, inversion on chromosome 8 [8135000–12000000], and inversion on chr 17 [40900000–45000000], GRCh37 build). We calculated 10 PCs for ancestry using SNPRelate version 1.16.0.³²

Genotyping for *APOL1*

Genotyping was performed using the Illumina Infinium® Expanded Multi-Ethnic Genotyping Array (MEGAEX). We excluded DNA samples: (1) with a call rate $<95\%$; (2) with inconsistently assigned sex; or (3) unexpected duplication. We performed whole genome imputation using the Michigan Imputation Server³³ with the Haplotype Reference Consortium,³⁴ version r1.1,^{34,35} as reference; we then filtered variants with (1) low imputation quality ($r^2 < 0.3$), (2) MAF $< 0.5\%$, and (3) variants with a MAF difference > 0.3 compared to the HRC reference panel.

Variants within *APOL1* were extracted from imputed genotype data. We used rs73885319 to define G1 and rs12106505 as a proxy for the G2 allele (rs71785313).³⁶ Individuals who were *APOL1* variant allele homozygotes or compound heterozygotes—defined as carriers of 2 copies of rs73885319 (G1/G1), 2 copies of rs12106505 (G2/G2), or 1 copy of each (G1/G2)—were considered to be high risk. Carriers of 1 or 0 *APOL1* risk alleles were considered low risk (i.e., a recessive model).^{5,36}

Statistical Analysis

Outcomes in patients with high-risk and low-risk *APOL1* genotypes were compared using logistic regression with adjustment for age at hospital admission, sex, and 3 PCs for ancestry. We performed further analyses 1) with additional adjustment for pre-existing severe renal disease (**Supplementary Table 2**) and 2) excluding patients with pre-existing severe renal disease (n=458) from the infection cohort.

In a secondary analysis we examined selected sepsis-related diagnoses previously reported to be associated with the *APOL1* high-risk genotype in a restricted cross-sectional PheWAS study.⁴ We used a similar approach and performed a cross-sectional PheWAS for the selected sepsis-related phenotypes (i.e., infection of internal prosthetic device, phecode 81; septicemia, phecode 38; sepsis, phecode 994.2, systemic inflammatory response syndrome [SIRS], phecode 994.1; and septic shock, phecode 994.21) in all EHR-reported Black patients in BioVU with MEGAEX genotypes (n=14,713). We identified phenotypes using phecodes, a phenotyping system based on ICD-9-CM and ICD-10-CM diagnosis codes.^{37,38} A phecode amalgamates related ICD codes mapping to a distinct disease or trait.^{37,38} A case was defined as an individual with 2 or more occurrences of the phecode of interest in the EHR. Controls were individuals without that code.

Individuals with 1 mention of the code or with related codes were excluded from the analysis to limit misclassification. We conducted logistic regressions with adjustment for age, sex, and 3PCs and repeated the analysis after excluding patients whose EHR contained one or more ICD codes indicating severe renal disease (**Supplementary Table 2**).

Chi-square tests were used to compare categorical characteristics and comorbidities between high- and low-risk *APOL1* genotype groups. T-tests were used to compare continuous characteristics. Logistic regression results are presented as odds ratios (ORs) and 95% confidence intervals (CIs); categorical variables are shown as number and percent; continuous variables are shown as median and interquartile range. P-values<0.05 were considered statistically significant.

Results

Infection cohort

The infection cohort included 2,242 Black patients hospitalized with an infection; 361 (16.1%) patients carried a high-risk *APOL1* genotype, and 1,881 (83.9%) carried low-risk genotypes (**Table 1**). The baseline characteristics of patients with the high- and low-risk genotypes did not differ significantly in age, sex, and most general medical comorbidities. However, renal-related comorbidities were significantly more frequent in the high-risk genotype group ($p=1.60\times 10^{-10}$) (**Table 1**).

Table 1.

Clinical characteristics of patients admitted to hospital with infections.

		Total (n=2242)	APOL1 low-risk group (n=1881)	APOL1 high-risk group (n=361)	p-value
Gender	Female, n (%)	1306 (58.3)	1096 (58.3)	210 (58.2)	1.00
	Male, n (%)	936 (41.7)	785 (41.7)	151 (41.8)	
Age (at admission), median (Q1-Q3)		50 (33-62)	50 (33-63)	50 (34-60)	0.67
Comorbidities*					
Congestive heart failure, n (%)		312 (13.9)	260 (13.8)	52 (14.4)	0.83
Chronic pulmonary disease, n (%)		378 (16.9)	320 (17)	58 (16.1)	0.72
Cerebrovascular disease, n (%)		221 (9.9)	197 (10.5)	24 (6.6)	0.03
Dementia, n (%)		39 (1.7)	33 (1.8)	6 (1.7)	1.00
Diabetes with chronic complication, n (%)		244 (10.9)	200 (10.6)	44 (12.2)	0.44
Diabetes without chronic complication, n (%)		170 (7.6)	136 (7.2)	34 (9.4)	0.18
Hemiplegia or paraplegia, n (%)		48 (2.1)	41 (2.2)	7 (1.9)	0.93
AIDS/HIV, n (%)		44 (2)	35 (1.9)	9 (2.5)	0.56
Malignancy, including lymphoma and leukemia, except malignant neoplasm of skin, n (%)		373 (16.6)	318 (16.9)	55 (15.2)	0.48
Myocardial infarction, n (%)		222 (9.9)	189 (10)	33 (9.1)	0.67
Mild liver disease, n (%)		38 (1.7)	33 (1.8)	5 (1.4)	0.78
Moderate or severe liver disease, n (%)		49 (2.2)	44 (2.3)	5 (1.4)	0.35
Peptic ulcer disease, n (%)		23 (1)	19 (1)	4 (1.1)	1.00
Peripheral vascular disease, n (%)		142 (6.3)	123 (6.5)	19 (5.3)	0.43
Renal disease, n (%)		545 (24.3)	409 (21.7)	136 (37.7)	1.60E-10
Rheumatic disease, n (%)		73 (3.3)	58 (3.1)	15 (4.2)	0.37
Metastatic solid tumor, n (%)		198 (8.8)	166 (8.8)	32 (8.9)	1.00

*Co-morbidities present in the preceding year as defined by criteria for the Charlson/Deyo comorbidity index.

Associations between high-risk *APOL1* genotype and sepsis

Within the infection cohort, 565 patients developed sepsis, including 105 (29.1%) with *APOL1* high-risk genotypes and 460 (24.5%) with low-risk genotypes. The risk of sepsis was significantly increased among patients with the high-risk *APOL1* genotypes (OR=1.29 [95% CI, 1.00–1.67; p=0.047]) (**Figure 1**). However, the association between sepsis and *APOL1* high-risk genotypes was not significant after adjustment for pre-existing severe renal disease (OR=1.14 [95% CI: 0.88-1.48; p=0.33]), nor after exclusion of those patients (n=458) with severe renal disease (OR=0.99 [95% CI, 0.70-1.39; p=0.95]) (**Figure 2**).

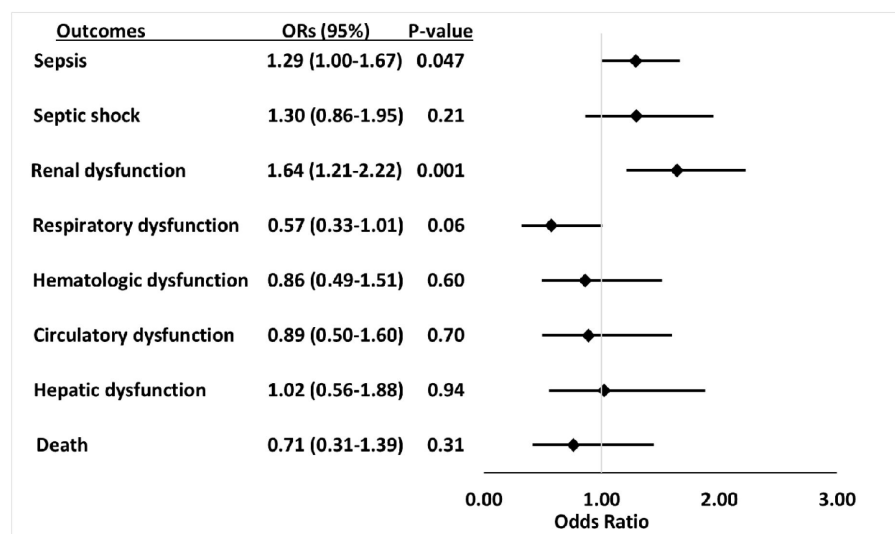


Figure 1.

Association between *APOL1* high-risk genotypes and the risk of sepsis, and septic shock, and individual sepsis organ dysfunction criteria.

Analyses were adjusted for age, sex and 3PCs. Sepsis was defined as meeting the Sepsis-3 criteria for sepsis,^{23,24} septic shock was defined using ICD9 and 10 codes for septic shock/severe sepsis (ICD-9-CM, 995.92 and 785.52; ICD-10-CM, R65.20

and R65.21); organ dysfunction data represent the individual organ dysfunction criteria in the Sepsis-3 definition; death was defined as in-hospital death or discharge to hospice.

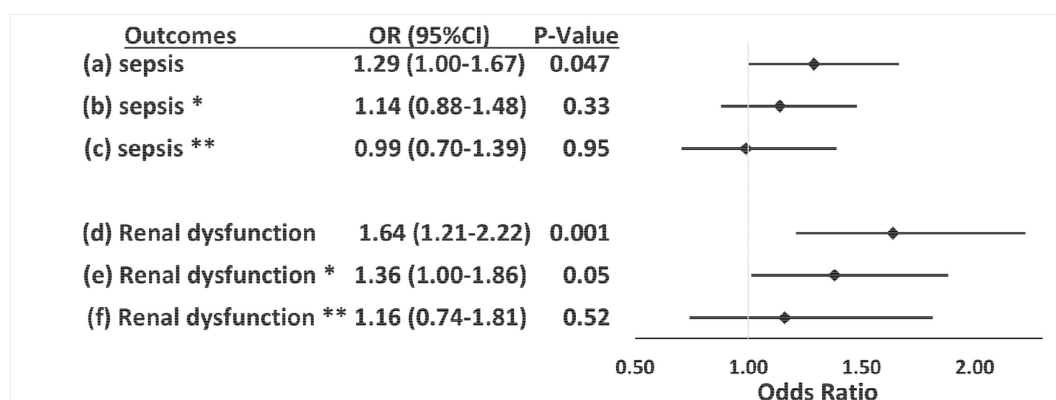


Figure 2.

Associations between *APOL1* high-risk genotypes and the risk of sepsis and renal dysfunction before and after adjustment for renal disease, and exclusion of patients with pre-existing severe renal disease.

We test the associations between *APOL1* high-risk genotypes and the risk of sepsis (a, b, c) or the risk of sepsis-related renal dysfunction (d, e, f). Sepsis was defined as meeting the Sepsis-3 criteria for sepsis,^{23,24} sepsis-related renal dysfunction represents the individual organ dysfunction criteria in the Sepsis-S3 definition. Analyses (a) and (d) were adjusted for age, sex, and 3 PCs. * In analyses (b) and (e) we adjusted for age, sex, 3 PCs, and pre-existing severe renal disease. **In analyses (c) and (f), we excluded patients with pre-existing severe renal disease and adjusted for age, sex, and 3 PCs.

Associations between the high-risk *APOL1* genotypes and components of sepsis or death

Among the 565 patients with sepsis, 163 (28.8%) had septic shock, 91 (16.1%) had cardiovascular dysfunction, 136 (24.1%) had respiratory dysfunction, 303 (53.6%) had renal dysfunction, 83 (14.7%) had hepatic dysfunction, 102 (18.1%) had hematologic dysfunction, and 84 (14.9%) died or were discharged to hospice. *APOL1* high-risk genotypes were significantly associated with renal dysfunction component of the Sepsis-3 criteria (OR=1.64 [95% CI, 1.21–2.22; $p=0.001$]), but they were not significantly associated with septic shock (OR=1.30 [95% CI, 0.86–1.95; $p=0.21$]) nor dysfunction of other organ systems (respiratory: OR=0.57 [95% CI, 0.33–1.01; $p=0.06$]; hematologic: OR=0.86 [95% CI, 0.49–1.51; $p=0.60$]; circulatory: OR=0.89 [95% CI, 0.50–1.60; $p=0.70$]; hepatic: OR=1.02 [95% CI, 0.56–1.88; $p=0.94$]), or death (OR=0.71 [95% CI, 0.31–1.39; $p=0.31$]) (**Figure 1, Supplementary Table 3**).

The association between high-risk *APOL1* genotypes and the renal dysfunction component of the Sepsis-3 criteria was markedly attenuated by adjusting for pre-existing severe renal disease present in the year before the index hospital admission (**Figure 2**, OR=1.36 [95% CI, 1.00–1.86; $p=0.05$]) and was nullified after the exclusion of patients with pre-existing severe renal disease (**Figure 2**, OR=1.16 [95% CI, 0.74–1.81; $p=0.52$]).

Cross-sectional associations between *APOL1* high-risk genotype and sepsis-related phenotypes

In the cross-sectional restricted PheWAS performed in Black participants in BioVU, *APOL1* high-risk genotypes were significantly associated with all prespecified sepsis-related phenotypes: infection of internal prosthetic device, OR=1.68 [95% CI, 1.32–2.13; $p=2.23\times 10^{-5}$]; systemic inflammatory response syndrome [SIRS], OR=1.49 [95% CI, 1.10–2.01; $p=9.87\times 10^{-3}$]; sepsis, OR=1.41 [95% CI, 1.18–1.67; $p=1.30\times 10^{-4}$]; septic shock, OR=1.51 [95% CI, 1.14–1.99; $p=3.89\times 10^{-3}$]; and septicemia, OR=1.30 [95% CI, 1.08–1.56; $p=6.01\times 10^{-3}$] (**Figure 3, Panel A**). However, the associations between *APOL1* and sepsis-related phenotypes were nullified after we excluded individuals with pre-existing severe renal disease (**Figure 3, Panel B**).

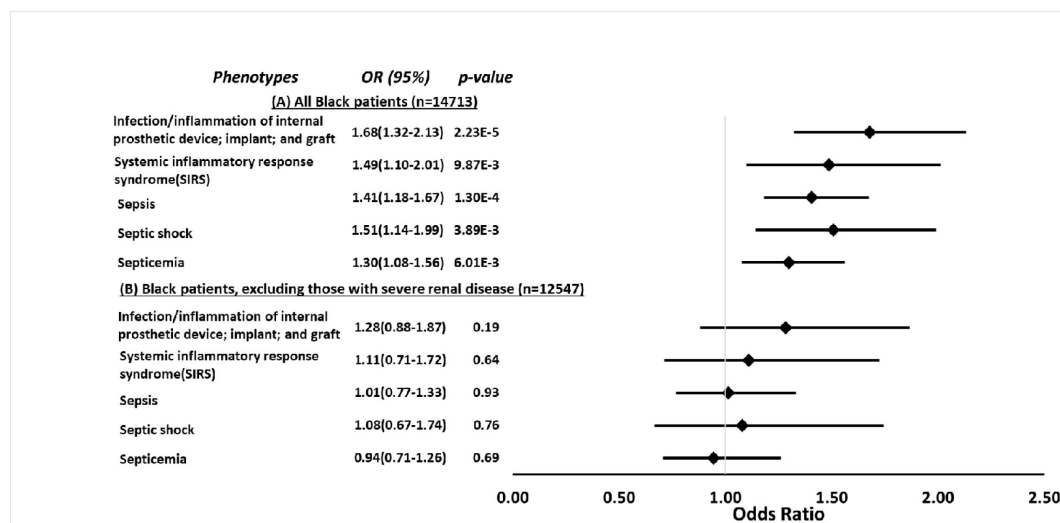


Figure 3.

Cross-sectional restricted PheWAS associations between *APOL1* high-risk genotype and sepsis-related phenotypes.

We tested the associations between *APOL1* high-risk genotype and sepsis-related phenotypes, including infection of internal prosthetic device, systemic inflammatory response syndrome [SIRS], sepsis, septic shock, and septicemia. We conduct the analyses in all EHR-reported Black individuals with available MEGAEX genotypes in BioVU (n=14,713, **panel A**) and after excluding individuals with severe renal diseases (n=12,547, **panel B**). Analyses were adjusted for age, sex, and 3 PCs.

Discussion

This study found that *APOL1* high-risk genotypes were significantly associated with an increased risk of sepsis in patients hospitalized with infections; however, this association was explained predominantly by the presence of pre-existing renal disease. Renal dysfunction was the only sepsis-associated organ dysfunction significantly associated with *APOL1* high-risk genotypes, and this risk was attenuated by adjustment for pre-existing renal comorbidity and nullified by the exclusion of patients with pre-existing severe renal disease.

Sepsis is a leading cause of death in the United States and the single most expensive diagnosis for Medicare, accounting for ~8% of all claims payments.^{24,39,40} There is a significant racial disparity in the risk of sepsis, and Black patients have a higher risk of sepsis and sepsis-related organ dysfunction relative to White patients.^{1,2} These factors have led to increased attention to understanding the mechanisms driving this disparity. A recent cross-sectional study performed in the MVP found that high-risk *APOL1* genotypes were associated with ~40% increased risk of sepsis compared to low-risk genotype patients, suggesting that functional genetic variants in the *APOL1* gene may contribute to the higher susceptibility to sepsis for Black patients compared to White patients.⁴ This relationship remained significant after adjustment for age, sex, and estimated glomerular filtration rate (eGFR). Additional mechanistic studies in animal models observed that *APOL1* is highly

expressed in the endothelium and that the high-risk variants were associated with increased inflammation, endothelial leakage, and sepsis severity.⁴ These findings raised the possibility that strategies to inhibit *APOL1* in patients who carry the high-risk variants may have therapeutic potential to prevent or ameliorate sepsis.

Our retrospective cohort study extends the findings of the MVP study, showing that among patients admitted to the hospital with infection, high-risk *APOL1* genotypes are indeed associated with the development of sepsis. However, the association between *APOL1* high-risk genotypes and sepsis is driven largely by the presence of pre-existing severe renal disease—a potent risk factor for infection, sepsis, and infection-related mortality.^{41,42} When we adjusted for pre-existing renal comorbidity, the association between *APOL1* high-risk genotypes and sepsis was attenuated, and when we removed patients with pre-existing severe renal disease from the analysis, the significant association was nullified.

Additionally, in a cross-sectional restricted PheWAS study of all Black participants in BioVU (a design similar to that of the MVP study), we found that *APOL1* high-risk genotypes were significantly associated with all prespecified sepsis-related phenotypes. However, those associations were not significant after excluding patients with severe renal disease. Three differences in study design may contribute to differences in the findings of the two studies. First, the retrospective cohort approach allowed us to better define the temporal relationship between comorbidities and sepsis and its organ dysfunction criteria. Second, we adjusted for renal disease rather than eGFR, because in the setting of dialysis or renal transplantation, the GFR estimated from a creatinine measurement may not capture the increased risk of sepsis in these patients. Third, we used a validated EHR algorithm rather than phecodes to define sepsis; nevertheless, a cross sectional analysis using phecodes was consistent with the primary analysis—*APOL1* high-risk genotypes were associated with sepsis-related phecodes; however, these associations were driven by those patients with pre-existing renal disease, a known consequence of *APOL1* high-risk genotypes.

Our retrospective cohort design using the Sepsis-3 criteria to define sepsis allowed us to study the role of *APOL1* high-risk genotypes in the progression from infection to sepsis and their relationship with specific sepsis organ dysfunction criteria. The findings showed that *APOL1* high-risk genotypes are associated with susceptibility to sepsis defined by Sepsis-3 criteria in patients hospitalized with infection, but this is not independent of pre-existing renal disease. It also showed that renal dysfunction was the only sepsis-related organ dysfunction affected by the presence of *APOL1* high-risk genotypes, suggesting that *APOL1* high-risk genotypes are not causal of sepsis beyond their association with renal disease and impaired renal function (which, in turn, increases susceptibility to sepsis). These findings more closely parallel those of another MVP study that excluded patients with severe pre-existing renal dysfunction and examined the effects of *APOL1* genotypes on outcomes among patients hospitalized with COVID-19 infections. In that study, high-risk *APOL1* genotypes were more strongly associated with acute kidney injury⁴³ than the need for mechanical ventilation or vasopressors. These results suggest that drug therapies in development to prevent and treat disease associated with *APOL1* high-risk genotypes might primarily affect the renal vulnerabilities that increase risk of sepsis, rather than prevention of progression to sepsis or acute treatment of sepsis generally.

Our observations further support the proposed 2-hit model,¹² in which genetic susceptibility (i.e., high-risk *APOL1* genotypes) combines with a trigger such as infection to cause disease (e.g., renal dysfunction). Indeed, a 3-hit model might be most appropriate for understanding the risk of sepsis, whereby high-risk *APOL1* genotypes are more likely to lead to acute renal dysfunction for patients in the settings of both infection and preexisting renal disease.

The current study offers several strengths. First, we identified patients progressing from infection to sepsis using a validated EHR algorithm. This approach also allowed us to

evaluate each organ dysfunction and its contribution to sepsis separately. Second, by leveraging the rich longitudinal EHRs, we were able to identify the pre-existing renal disease and assess its effect on sepsis. Third, we performed analyses that both included and excluded patients with pre-existing chronic severe renal disease, allowing us to better define the contribution of pre-existing renal disease to the development of sepsis in the setting of *APOL1* high-risk genotypes.

We also acknowledge the study's limitations, primarily related to a retrospective cohort study using EHR information. First, ascertainment of comorbidities was based on ICD codes, and these may not completely reflect comorbidities. Second, there are many factors that affect health (e.g., alcohol use, diet, and lifestyle) that are not captured well in the EHR but could impact susceptibility to sepsis; however, there is no reason to expect that such factors are differentially distributed according to genotype. Third, we defined death as short-term mortality, including in-hospital death and discharge to hospice;²⁶ this definition may underestimate the true mortality rate due to sepsis. Fourth, we did not include patients with concurrent COVID-19 infections because their clinical manifestations and genetic predispositions might differ from that of patients who develop sepsis after presumed bacterial infection. Last, a larger cohort of Black patients with infection and sepsis could potentially provide more power such that the confidence intervals around the point estimates were smaller, more definitively excluding the possibility of clinically important differences in outcomes.

In conclusion, in this cohort of Black participants hospitalized with infection, *APOL1* high-risk genotypes were associated with an increased risk of sepsis; however, this increased risk was attributable predominantly to pre-existing renal disease. Further, renal dysfunction was the only sepsis-associated organ dysfunction associated with *APOL1* high-risk genotypes.

Data Availability

All data produced in the present study are available upon reasonable request to the authors

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The first and corresponding authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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The funders had no role in design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Contributor Statement

L.J., G.L., A.O., A.I., A.L.D., L.L.D., C.P.C., N.C., W.Q.W., C.M.S. and Q.F. wrote the manuscript; L.J., C.M.S., and Q.F. designed the research; L.J., G.L., A.O., A.I., A.L.D., L.L.D., C.P.C., W.Q.W., C.M.S. and Q.F. performed the research; L.J., G.L., C.M.S and Q.F. analyzed the data.

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Joint Public Review:

The study has main limitations which need to be addressed and there is lack of functional explanation of carriage. These limitations are: a) the lack of inclusion of non-Black patients; and b) the lack of appropriate explanation if results are false-positive since APOL1 provides risk for chronic renal disease (CRD) and patients with CRD are predisposed to sepsis. Sepsis occurred in 565 Black subjects, of whom 105 (29%) had APOL1 high-risk genotype and 460 had-low risk genotype. Importantly, the risk for sepsis associated with APOL1 HR variants was no longer significant after adjusting for subjects pre-existing severe renal disease or after excluding these subjects. Thus, the susceptibility pathway seems to be: APOL1 variants > CKD > sepsis diathesis.

Suggestions to the authors:

- The authors need to provide analysis of patients of non-Black origin.
- The Table of demographics needs to include the type of infections and the underlying pathogen.
- The authors need to provide convincing analysis if results are false-positive since APOL1 provides risk for chronic renal disease (CRD) and patients with CRD are predisposed to sepsis. For this purpose, they have to provide evidence if the sepsis causes (both type of infection and implicated pathogens) in patients with CRD who are carriers of APOL1 variants are different than in patients with CRD who are not carriers of APOL1 variants.
- Why concentrations of APOL1 were not measured in the plasma of patients?
- Why analysis towards risk for death is not done?
- The authors need to explain why functional information is not provided.
- n 162-172: too many assumptions have been used for the trial; thus, progression to sepsis is difficult to define. According to Sepsis-3 sepsis is no more a continuum from infection to sepsis and septic shock. Some patients presented with sepsis (-1, 0, 1 days considered by the authors) and when electronic health records are used, we are not able to detect the exact timepoint of SOFA score turning to a 2-point increase. This is a major limitation of the

methodology presented.

Same applies for all comorbidities and data extracted from electronic health records.

- P value significance thresholds were set at 0.05, except for the PWAS where the threshold was set at 0.05/5 (p13). It would be helpful to list at this point what the 5 outcomes were that led to this adjusted threshold.

"Risk of sepsis was significantly increased among patients with high-risk genotypes (OR 1.29, 1.0 to 1.67, P1.29, CI 1.00-1.67, $P < 0.47$)."

Some would argue that a confidence interval that includes 1.0 indicates non-significance.

- The Discussion is too long and should be shortened.