

Association between *APOL1* risk variants and the occurrence of sepsis in patients hospitalized with infections

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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 occurrence of either sepsis or sepsis-related phenotypes in patients hospitalized with infections, independent of their association with pre-existing severe renal disease.

Objective

To examine the association between *APOL1* high-risk genotypes and the risk of sepsis and sepsis-related phenotypes in patients hospitalized with infections.

Design, setting, and participants

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

Exposures

Carriage of *APOL1* high-risk genotypes.

Main outcomes and measures

The primary outcome was sepsis; secondary outcomes were short-term mortality 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 short-term mortality. 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 severe 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) found that variants in the apolipoprotein L1 gene (*APOL1*), common in people of African ancestry, are associated with sepsis incidence and severity.⁽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 *APOL1* has a direct association with sepsis. More specifically, since *APOL1* is associated with renal disease^(9, 17–19) and existing renal disease is associated with worse sepsis outcomes⁽²⁰⁾ (including increased kidney injury), the relationship between *APOL1* and sepsis external to existing renal disease remains unclear. Further, it is important to assess whether other organ system dysfunctions typical of sepsis (i.e., hepatic, respiratory, circulatory, and hematologic dysfunction) are associated with *APOL1* to appropriately define the causal pathway between *APOL1* and sepsis. This determination of association could help guide the viability of implementing treatment options for sepsis using therapies that target APOL1. In particular, several inhibitors of APOL1 are in various stages of development—if *APOL1* high-risk genotypes are directly associated with the pathogenesis of sepsis external to pre-existing severe renal disease or other organ system dysfunction, such drugs could be offered to high-risk patients to prevent or treat the occurrence of sepsis in scenarios of acute infection.⁽²¹⁾

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 sepsis for patients hospitalized with an infection, particularly independent of the association between the genotypes and 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.

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 to assess the association between carriage of *APOL1* high-risk alleles and the occurrence of sepsis before and after consideration of pre-existing severe renal disease. Additionally, to replicate findings from a previously published MVP sepsis study,⁽⁴⁾ we performed a restricted phenome-wide association analysis (PheWAS) in all Black patients with existing genotypes; we determined the association between carriage of *APOL1* high-risk genotypes and phenotypes previously reported to be associated with sepsis, further expanding the analysis to account for pre-existing severe renal disease based upon the primary analysis of this study.⁽⁴⁾ This study was reviewed by the VUMC Institutional Review Board; given the study's retrospective design and use of deidentified data only, informed consent was waived.

Inclusion/Exclusion Criteria

The primary 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.⁽²²⁾ We selected patients with EHR-reported Black race only (i.e., as reported by the patient or, secondarily, by a provider) because *APOL1* high-risk genotypes are virtually exclusive to populations of recent African ancestries,^(11, 14, 23, 24) the majority of whom are identified as having Black race in our dataset (i.e., EHR-reported race is highly consistent with genetic ancestry in BioVU).⁽²⁵⁾ This restriction helps control for factors associated with socially-determined race which may affect individuals' sepsis outcomes external to genetic ancestry (e.g., an individual with predominantly African genetic ancestry who identifies as having White race). To account for genetic ancestral diversity within the cohort, all analyses were adjusted for principal components for ancestry (see below). The day of hospital admission was designated day 0. International Classification of Disease, ninth revision, Clinical Modification (ICD-9-CM); tenth revision (ICD-10-CM); Current Procedural Terminology (CPT) codes; medications; labs; and clinical notes were used for cohort construction and covariates. 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).^(22, 26, 27) We used ICD-9-CM and ICD-10-CM codes for this definition of infection based on the criteria of Angus *et al.*,^(28, 29) excluding viral, mycobacterial, fungal, and spirochetal infections, as we have described in detail previously.^(22, 26, 27) 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.^(22, 26, 27)

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 (i.e., renal, hepatic, respiratory, circulatory, and hematologic dysfunction) as well as severe sepsis/septic shock, and short-term mortality.

Sepsis was defined by the Sepsis-3 criteria of concurrent infection and organ dysfunction (**Supplementary Figure 1**)^(29, 30) using the EHR definition that was developed in real-world hospital settings^(30, 31) and optimized, validated, and applied across EHR systems from 409

hospitals.(30) The algorithm uses billing codes and clinical criteria, with a specificity of 98.1% and a sensitivity of 69.7%.(30) We further adapted (i.e., included relevant ICD-10-CM codes) and then applied the EHR-based Sepsis-3 algorithm to the de-identified EHRs in our system, as described previously.(26, 27) Because the vast majority of community acquired sepsis cases (87%) are present on admission to hospital,(30) 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 previously validated this updated algorithm in our EHRs.(22, 26, 27)

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%),(30) or they met any Sepsis-3 criterion for serious organ dysfunction (**Supplementary Figure 1**).(30) Criteria for organ system dysfunction included: (1) *circulatory*: a) use of a vasopressor, which we extracted as use of levophed (norepinephrine), or b) use of the vasopressors (i.e., dobutamine or dopamine) unrelated to stress echocardiography (CPT codes 78452, 93015, 93018, 93016, 93017, and 93351 within days -1, 0, and 1) and with ≥ 2 mentions of any of the keywords (i.e., “infection,” “sepsis,” or “septic”); (2) *respiratory*: ICD or CPT codes for ventilation and admission to an ICU; (3) *renal*: 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*: 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*: a platelet count $<100,000/\mu\text{L}$ 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).(22, 26, 27) Short-term mortality was defined as patients who 1) had death recorded in the EHR within the index hospital stay or 2) were discharged to hospice.(32)

Covariates

We extracted demographic characteristics from the EHRs, including sex and age at the time of the index hospital admission, as well the types of infection at the time of hospitalization (**Supplementary Table 1**). Comorbidities were collected(33, 34) using relevant diagnostic codes in the year before the index hospital admission (**Supplementary Table 2**) grouped into the 17 Charlson/Deyo comorbidity categories.(35–37) 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 3**). 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.(38)

Genotyping for *APOL1*

Genotyping was performed using the Illumina Infinium® Expanded Multi-Ethnic Genotyping Array (MEGA^{EX}). We excluded DNA samples: (1) with a call rate $<95\%$; (2) with inconsistently assigned sex; or (3) that were unexpected duplicates. We performed whole genome imputation using the Michigan Imputation Server(39) with the Haplotype Reference Consortium,(40) version r1.1,(40) (41) as reference; we then filtered variants with (1) low imputation quality ($r^2 < 0.3$), (2) MAF $< 0.5\%$, and (3) MAF absolute difference > 0.3 when 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).⁽¹⁷⁾ 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, 17)

Statistical Analysis

Primary and secondary 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 3**), 2) excluding patients with pre-existing severe renal disease (n=458) from the infection cohort, and 3) including only patients with pre-existing severe renal disease.

In the replication analysis, we examined selected sepsis-related diagnoses previously reported to be associated with the *APOL1* high-risk genotype in a restricted PheWAS study.⁽⁴⁾ We used a similar approach and performed a restricted 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 MEGA^{EX} genotypes (n=14,713). We identified phenotypes using phecodes, a phenotyping system based on ICD-9-CM and ICD-10-CM diagnosis codes.^(42, 43) A phecode amalgamates related ICD codes mapping to a distinct disease or trait.^(42, 43) 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, as an expansion of the original approach,⁽⁴⁾ repeated the analysis after excluding patients whose EHR contained one or more ICD codes indicating pre-existing severe renal disease (**Supplementary Table 3**).

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, except the restricted PheWAS for which p-values<0.01 (.05 divided by 5 phecodes [namely, infection of internal prosthetic device, septicemia, sepsis, SIRS, and septic shock]) were considered significant. All analyses were conducted using R version 4.1.0.

Results

The primary 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 and infection types. However, renal-related comorbidities were significantly more frequent in the high-risk genotype group ($p=1.60 \times 10^{-10}$) (**Table 1**).

		Total (n=2,242)	APOL1 low-risk group (n=1,881)	APOL1 high-risk group (n=361)	p-value
Gender	Female, n (%)	1,306 (58.3)	1,096 (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

Table 1.

Clinical characteristics of patients admitted to the hospital with infections

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
Infection Type**				
Circulatory, n (%)	35 (1.6)	29 (1.5)	6 (1.7)	0.86
Digestive, n (%)	247 (11)	217 (11.5)	30 (8.3)	0.07
Genitourinary, n (%)	769 (34.3)	648 (34.5)	121 (33.6)	0.75
Intestinal, n (%)	60 (2.7)	46 (2.4)	14 (3.9)	0.12
Musculoskeletal, n (%)	109 (4.9)	92 (4.9)	17 (4.7)	0.89
Neurologic, n (%)	43 (1.9)	35 (1.9)	8 (2.2)	0.65
Other bacterial, n (%)	879 (39.3)	723 (38.5)	156 (43.3)	0.08
Respiratory, n (%)	711 (31.8)	589 (31.3)	122 (33.9)	0.34
Skin, n (%)	325 (14.5)	286 (15.2)	39 (10.8)	0.03
Other, n (%)	322 (14.4)	268 (14.3)	54 (15)	0.72

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

** Total n=2,239; APOL1 low-risk group n=1,879; APOL1 high-risk group n=360

Table 1. (continued)

Associations between high-risk *APOL1* genotype and sepsis

Within the primary cohort of patients hospitalized with infections, 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). We also found no association between sepsis and *APOL1* high-risk genotypes in analysis of patients with severe renal disease alone (OR=1.29, [95% CI, 0.84–1.98, $p=0.25$]).

Associations between the high-risk *APOL1* genotypes and components of sepsis or short-term mortality

For secondary outcomes, 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 short-term mortality (OR=0.71 [95% CI, 0.31–1.39; $p=0.31$]) (Figure 1, Supplementary Table 4).

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; $p=0.52$]). We also found no association between *APOL1* high-risk genotypes and the renal dysfunction criterion in analysis of patients with severe renal disease alone (OR=1.43, [95% CI, 0.90–2.26, $p=0.13$]).

Associations between *APOL1* high-risk genotype and sepsis-related phenotypes

Using a parallel methodology, the restricted PheWAS performed in Black participants in BioVU ($n=14,713$, Supplementary Table 5) replicated the association of *APOL1* high-risk genotypes and all prespecified sepsis-related phenotypes previously identified as associated with *APOL1* high-risk genotypes in an MVP cohort: 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, in an expansion of the original approach, the associations between *APOL1* and sepsis-related phenotypes were nullified after we excluded individuals with pre-existing severe renal disease ($n=2,166$) and reran the analyses ($n=12,547$, Figure 3, Panel B).

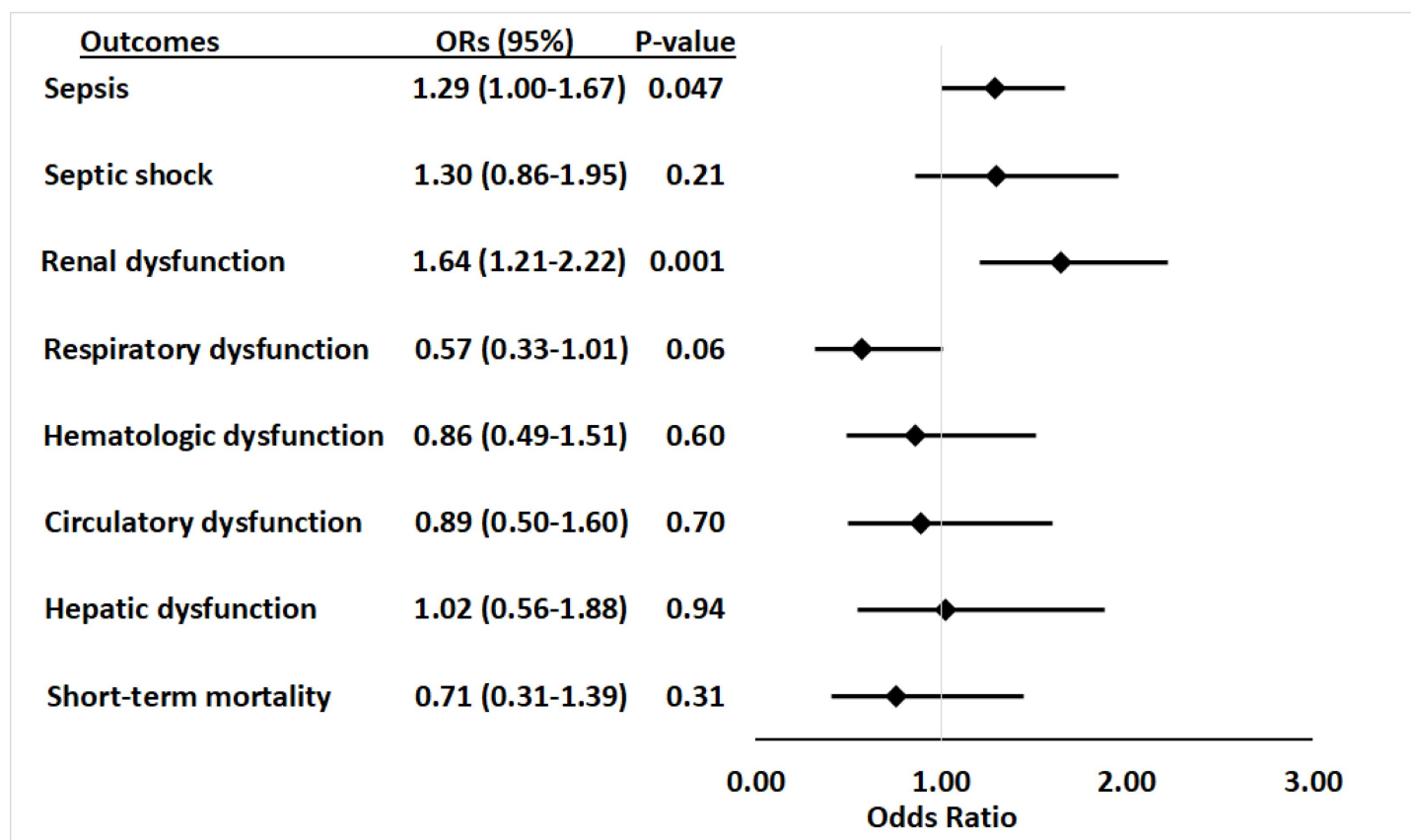


Figure 1.

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 3 principal components (PCs). Sepsis was defined as meeting the Sepsis-3 criteria for sepsis,^(29, 30) 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; short-term mortality was defined as in-hospital death or discharge to hospice.

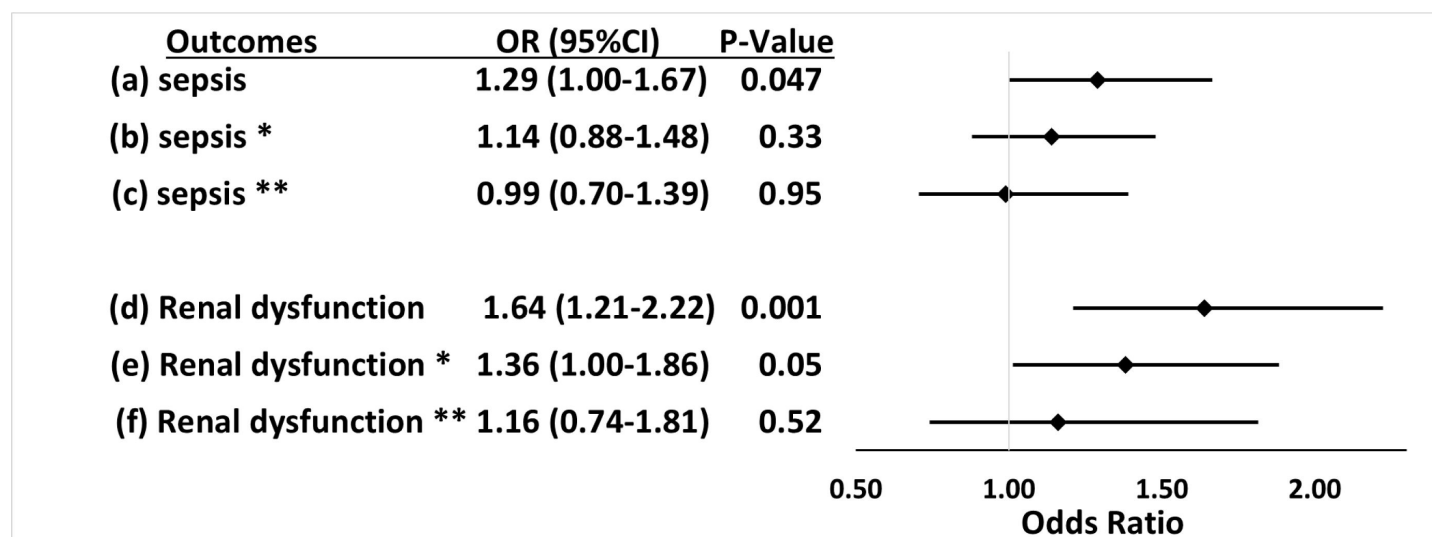


Figure 2.

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,^(29, 30) 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 principal components, 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 principal components.

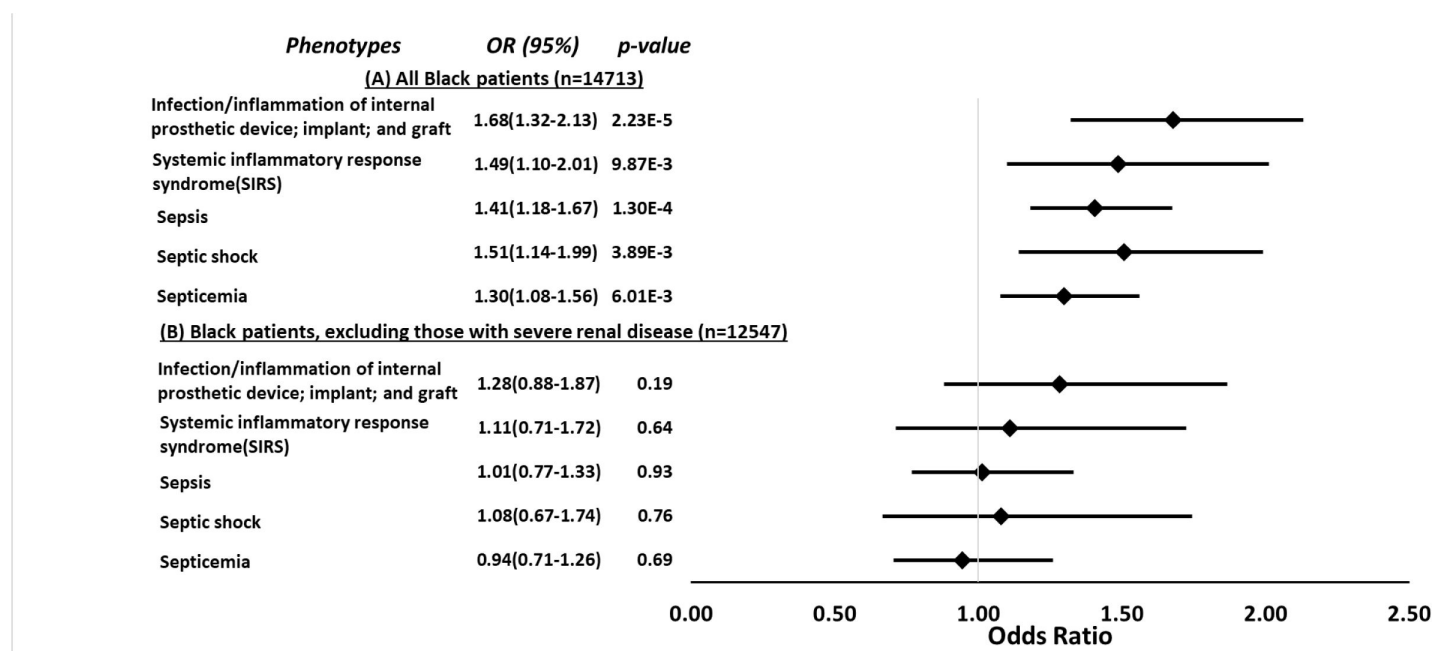


Figure 3.

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 MEGA^{EX} 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 principal components.

This retrospective cohort 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 severe 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 severe renal comorbidity and nullified by the exclusion of patients with pre-existing severe renal disease. Moreover, there appeared to be no increased risk of sepsis for carriage of high-risk genotypes among patients with pre-existing severe renal disease.

Our findings of an overall increased sepsis risk are consistent with a recent cohort study performed in the MVP which found that high-risk *APOL1* genotypes were associated with ~40% increased risk of sepsis compared to low-risk genotype patients, remaining significant after adjustment for age, sex, and estimated glomerular filtration rate (eGFR).^(44, 45) Additional mechanistic analysis in this study 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.⁽⁴⁶⁾ These findings raised the possibility that strategies to inhibit *APOL1* in patients who carry the high-risk variants may have therapeutic potential to prevent occurrence or ameliorate symptoms of sepsis.

This study extends and refines the findings of the MVP study, showing an association, if modest, between high-risk *APOL1* genotypes and the occurrence of sepsis among patients admitted to the hospital with infections. 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.^(44, 45) 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 the restricted PheWAS of all Black participants in BioVU (a design parallel 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, the replication 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 severe renal disease, a known consequence of *APOL1* high-risk genotypes.

The lack of association independent of pre-existing severe renal disease suggests that *APOL1* high-risk genotypes are not acutely 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 injury⁽⁴⁹⁾ than the need for mechanical ventilation or vasopressors. These results suggest that drug therapies in development to prevent and treat

diseases associated with *APOL1* high-risk genotypes might primarily affect the renal vulnerabilities that increase risk of sepsis, rather than immediate prevention of sepsis or acute treatment of sepsis once hospitalized.

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 pre-existing severe renal disease and assess its effect on the risk of developing sepsis. Third, we performed analyses that included, excluded, and exclusively focused on patients with pre-existing chronic severe renal disease, allowing us to more completely define the contribution of *APOL1* high-risk genotypes to the development of sepsis.

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;⁽³²⁾ 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. Fifth, as we did not find an association after adjustment for pre-existing severe renal disease, we did not perform any additional functional analysis. 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; a larger cohort could also provide the opportunity for additional granularity in analysis (e.g., stratified by whether patients are on dialysis).

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 severe 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

Acknowledgements

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.

Author Contributions

L.J., G.L., A.O., A.I., A.L.D., L.L.D., A.M.H., C.P.C., N.J.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 many limitations which need to be addressed and there is a 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.

Author Response

The following is the authors' response to the original reviews.

Thank you for the helpful comments regarding our manuscript, "Association between APOL1 risk variants and the occurrence of sepsis among patients hospitalized with infections." We have revised the title of the manuscript in response to reviewer comments. Additionally, we have updated the manuscript with analyses among patients with pre-existing renal disease alone as well as other items suggested by the reviewers. The Tables have been renumbered to accommodate these revisions.

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.*

We apologize for not fully clarifying that the APOL1 high-risk genotypes are virtually exclusive to populations of recent African ancestries,^{1–4} the majority of whom are identified as having Black race in our dataset.⁵ To illustrate the rarity of APOL1 high-risk genotypes in other reported races, we examined the frequency of these genotypes in White patients who had been hospitalized with infections at VUMC (comparable to the cohort of Black patients used in the study). Compared to the 361 out of 2242 (16.1%) Black patients hospitalized with infections carrying APOL1 high-risk genotypes, there were only 8 carriers of APOL1 high-risk genotypes out of 12,990 White patients (0.06%); of these 8, 2 patients developed sepsis during hospitalization. Due to a low number of carriers (n=8) and limited number of events (n=2), we

could not proceed with further analysis. Patients reported as other races (e.g., Asian and American Indian) are less frequent than White or Black patients in the VUMC de-identified EHR; as such, we would anticipate similarly small, if any, numbers of high-risk genotypes among these groups, with insufficient power for meaningful analysis. Comparisons between racial groups that did not have carriage of the APOL1 high-risk genotypes would increase the possibility of confounding by factors associated with racial identity (e.g., social determinants of health), rather than genotype; as such, detected differences would likely reflect those factors, rather than the impact of APOL1.

We have now added clarifying language in the Methods section.

• *The Table of demographics needs to include the type of infections and the underlying pathogen.*

Microbiological evidence of specific infection types is not available for the majority of records for patients hospitalized with infections (as well as sepsis); indeed, for many patients with common infections (e.g., pneumonia) the pathogen is often not identified.⁶ While we do not have details regarding the underlying pathogens, we were able to determine infection categories at admission. We now include details regarding the categories of infection based on ICD codes in Supplementary Table 1, and the updated Table 1 now includes that information for the APOL1 high-risk and low-risk groups. Given that individuals could have more than one type of infection, we also tested the number of types of infection and found no significant difference between the high-risk and low-risk genotypes ($p=0.77$).

• *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.*

Indeed, we believe the presented findings suggest that the apparent association between APOL1 high-risk genotypes and sepsis is driven by associated pre-existing severe renal disease rather than APOL1 itself; we appreciate the suggestion to conduct additional analyses to assess whether APOL1 high-risk genotypes impact the occurrence of sepsis among those patients with pre-existing severe renal disease. We note that this analysis could also be biased towards detecting a spurious association between APOL1 high-risk genotypes and sepsis if, within the subgroup with pre-existing severe renal disease, patients with high-risk genotypes also have more severe pre-existing renal disease.

Among the patients with pre-existing severe renal disease ($n=458$), 121 (26.4%) were carriers of the APOL1 high-risk genotypes. First, we assessed the severity of renal disease among these patients, detecting an association between APOL1 high-risk genotypes and greater severity (i.e., CKD stage 5/ESRD) when adjusted for age, sex, and 3 PCs: OR=2.29 (95% CI, 1.42-3.67, $p=6.25 \times 10^{-4}$). Then, we compared the primary outcome of sepsis in patients with APOL1 high-risk and low-risk genotypes for this subgroup. Despite the potential bias toward detecting an association between sepsis and the high-risk genotype based on the severity of pre-existing renal disease, there was no significant association between the high-risk genotypes and sepsis (OR=1.29, [95% CI, 0.84-1.98, $p=0.25$]). Finally, we assessed infection categories (as described in the above response) in this subgroup. We found no significant differences between the high-risk and low-risk genotypes in the frequency of any infection category.

These results suggest that the APOL1 high-risk genotypes are not associated with an increased risk of sepsis among patients who have pre-existing severe renal disease. Taken with our other findings, the high-risk genotypes appear to have little or no association with sepsis beyond their association with renal disease. As such, drugs targeting those genotypes would

likely have little effect in the acute setting of hospitalization with infection; rather, their primary contribution to the prevention of sepsis would need to target the prevention of underlying renal disease. We have revised our Methods, Results, and Discussion to include these findings.

- *Why concentrations of APOL1 were not measured in the plasma of patients?*

Although APOL1 high risk genetic variants have been repeatedly associated with renal-related clinical phenotypes, and many candidate mechanisms have been proposed,⁴ there has been contradictory evidence regarding whether the genetic variants could be linked to altered plasma APOL1 levels or whether APOL1 levels are related to elevated risk of renal disease. This is not surprising since it is the altered biological function of the APOL1 structural variant that is important, rather than the concentration of APOL1 protein. While some studies have detected an association between APOL1 high-risk genotypes and plasma levels among patients with renal dysfunction and sepsis,⁷ other population studies have suggested no association between APOL1 plasma levels and renal function.⁸ Plasma APOL1 levels are seldom measured in clinical practice and thus were not available in this retrospective cohort. However, given the inconsistency of findings and the underlying biology of APOL1, we believe measurements of levels (rather than function) is unlikely to be illuminating.

- *Why analysis towards risk for death is not done?*

In the current study, we focused on the risk of in-hospital death. We did not include the risk of out-of-hospital death due to potential data fragmentation. Specifically, we only have access to the patient's EHRs at VUMC, and death after hospital discharge is not always included in a patient's EHR unless relatives contact the hospital. As such, we focused on in-hospital death, which we validated previously with manual chart review.⁹ Paralleling the design from a previous publication assessing sepsis outcomes, we included discharge to hospice as part of our in-hospital death algorithm,¹⁰ as patients with a terminal illnesses are often discharged to hospice. However, to clarify this outcome component, we now refer to in-hospital deaths and discharge to hospice collectively as "short-term mortality." In this study, of the 84 total patients with the "short-term mortality" outcome, 47 patients were in-hospital deaths and 37 patients were discharged to hospice. Parallel to the short-term mortality, we found no association with in-hospital death alone. Ln 190: discharge to hospice. I am not sure this can be translated in in-hospital mortality. As noted in the above response, we have rephrased this outcome component as "short-term mortality," following the design of a previous publication assessing sepsis outcomes.¹⁰

- *The authors need to explain why functional information is not provided.*

Functional studies were not performed for several reasons. Animal models are problematic because mice do not have an ortholog to the human APOL1 gene, and the various models developed all have limitations, particularly when second and third perturbations (sepsis and renal impairment) would need to be introduced.¹¹ Also, since we did not observe an association between the genotypes and sepsis independent of pre-existing severe renal disease, we did not pursue additional functional studies. We do describe existing functional analysis in the introduction and briefly in our discussion; we now note this limitation.

- *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.

Thank you for highlighting this issue. We acknowledge that our choice of wording was unclear. The choice of ICD infection codes during the initial hospitalization window (i.e., -1, 0, 1 days) was aimed to generate a clean cohort of patients hospitalized with infections (i.e., not secondary infections or development of sepsis after an in-hospital procedure), rather than to establish a timeline of progression from infection to sepsis. As you correctly note, our algorithm would capture patients presenting with infection and concurrent sepsis at admission rather than progression to sepsis, and the exact timepoint of the SOFA score meeting the 2-point criterion is difficult to capture through the EHR. Accordingly, we conducted no time-dependent analysis in the current study. To more accurately convey the methodology of the current study (i.e., testing the association between APOL1 high-risk genotypes—which the patients were born with—and the risk of sepsis for patients hospitalized with infections), we revised the manuscript thoroughly, replacing “progression to sepsis” with “occurrence of sepsis” in the title, abstract as well as on pages 7, 8, and 19. We also acknowledge the limitations of using EHR in the Discussion.

• 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.

We have revised the manuscript accordingly.

"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.

While the lower bound of the confidence interval appears to meet the 1.0 threshold with only 2 decimal places (which would preclude significance), when taken to the 4th decimal place, the value is 1.0037, demonstrating that the 95% CI did not meet or cross under the 1.0 threshold, and thus the odds ratio should be considered significant (as evidenced by the $p=0.047$). This result is consistent with other studies that have detected an association between the high-risk genotypes and sepsis,⁷ but you correctly note that readers can discern from the confidence intervals that the finding is not strong.

• The Discussion is too long and should be shortened.

We have revised the Discussion.

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