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Article

Causal Relationship between Mitochondrial-Associated Proteins and Sepsis in ICU Patients: A Mendelian Randomization Study

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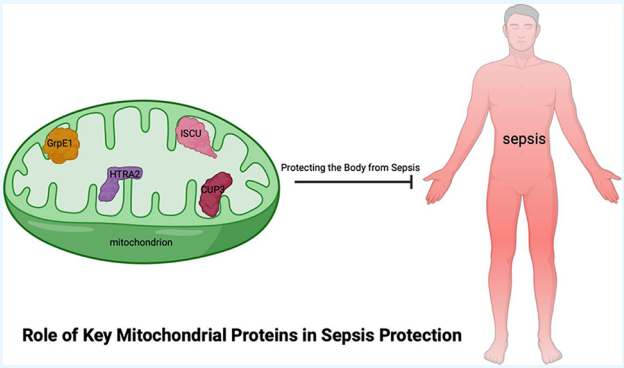
ABSTRACT: Background: The alarming mortality rate of sepsis in ICUs has garnered significant attention. The precise etiology remains elusive. Mitochondria, often referred to as the cellular powerhouses, have been postulated to have a dysfunctional role, correlating with the onset and progression of sepsis. However, the exact causal relationship remains to be defined. Method: Employing the Mendelian randomization approach, this study systematically analyzed data from the IEUOpenGWAS and UKbiobank databases concerning mitochondrial function-related proteins and their association with sepsis, aiming to delineate the causal relationship between the two. Results: The findings underscored a statistically significant association of GrpE1 with sepsis, registering a *P* value of 0.005 and an OR of 0.499 (95% CI: 0.307−0.810). Likewise, HTRA2, ISCU, and CUP3 each manifested significant associations with sepsis, yielding OR values of 0.585, 0.637, and 0.634, respectively. These results suggest potential implications of the aforementioned proteins in the pathogenesis of sepsis. Conclusion: The present study furnishes novel evidence elucidating the roles of GrpE1, HTRA2, ISCU, and CUP3 in the pathophysiology of sepsis. Such insights pave the way for a deeper understanding of the pathological mechanisms underpinning sepsis and hint at promising therapeutic strategies for the future.



INTRODUCTION

Sepsis is a lethal affliction with far-reaching and alarming implications.[1,2] Statistics reveal that even in the absence of complications, its mortality rate is a staggering 25%, which soars to 80% in cases of multiorgan failure, becoming the leading cause of death in ICU settings.[3−5] Faced with this grave challenge, we must not turn a blind eye but delve deeply into its etiology and mechanisms, seeking viable solutions. The objective of this study is precisely to unravel the intricate link between sepsis and mitochondrial function, examining its profound impact on human health.

Since the 1970s, the inter-relation of mitochondria and sepsis has been at the academic forefront. However, despite decades of rigorous research, the exact role and mechanism of mitochondria in sepsis remain elusive. Early investigations were predominantly centered on rat models, where it was discerned that septic shock could suppress mitochondrial functions.[6,7] This revelation highlighted the pivotal role of mitochondria in the onset and progression of sepsis. In recent years, with the advancement of scientific methodologies, our comprehension of the nexus between mitochondria and sepsis has deepened. Numerous contemporary studies indicate that the role of mitochondria in sepsis extends beyond mere energy metabolic imbalances.[8] For instance, oxidative stress within mitochondria can escalate reactive oxygen species production, leading to redox imbalance, and subsequently altering cellular physiological conditions.[9] Moreover, some research posits that transient mitochondrial suppression might be a cellular protective strategy, aiding cell survival when energy demands wane.[10,11] Despite continuous research endeavors, a comprehensive and systematic theoretical framework elucidating the causal link between mitochondrial function and septicemia in ICU patients has yet to be established.

Mendelian randomization, an epidemiological approach, employs genetic variations as instrumental variables (IVs) to ascertain causal relationships between exposures and outcomes.[12] The strength of this technique lies in its ability to mitigate confounding effects, address reverse causation, and its reliance on large sample studies ensures more robust results.[13] Hence, this study aims to employ Mendelian randomization to explore whether a causal link exists between mitochondrial exposure and sepsis in ICU patients and, if so, the biological

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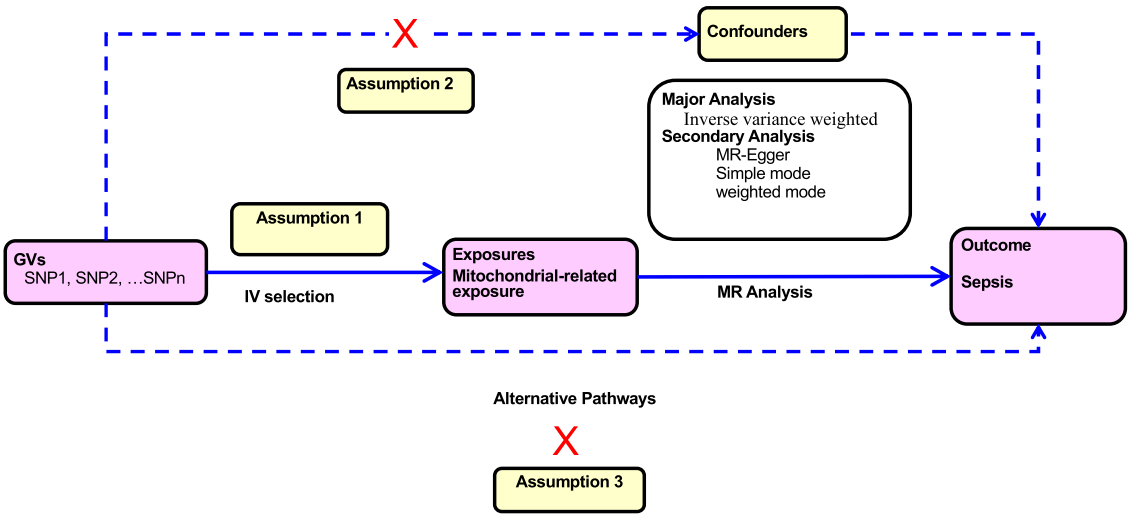

Figure 1. Directed acyclic graphs for classical Mendelian randomization designs. The arrows denote causal relations between two variables, pointing from the cause to the effect. The causal pathway is blocked if "X" is placed in the arrowed line. MR, Mendelian randomization.

Table 1. Instrumental Variables Used in MR Analysis of the Association between Mitochondrial-Related Proteins and Sepsis

exposure	method	no. SNP	*P* value	OR	95%CI	pleiotropy	heterogeneity	*F*
GrpE1[a]	inverse variance weighted	7	0.005	0.499	0.307−0.810	0.495	0.636	23.601
	MR Egger		0.636	0.742	0.232−2.372			
	simple mode		0.096	0.368	0.136−0.992			
	weighted mode		0.094	0.372	0.141−0.985			
HTRA2[a]	inverse variance weighted	9	0.018	0.585	0.376−0.911	0.877	0.374	24.934
	MR Egger		0.404	0.524	0.126−2.182			
	simple mode		0.719	0.839	0.333−2.113			
	weighted mode		0.754	0.853	0.328−2.223			
ISCU[a]	inverse variance weighted	12	0.018	0.637	0.438−0.926	0.966	0.379	22.393
	MR Egger		0.397	0.649	0.249−1.691			
	simple mode		0.533	0.736	0.289−1.873			
	weighted mode		0.496	0.742	0.323−1.705			
MCE[a]	inverse variance weighted	13	0.040	0.682	0.474−0.982	0.285	0.877	24.761
	MR Egger		0.813	1.124	0.437−2.890			
	simple mode		0.642	0.828	0.382−1.798			
	weighted mode		0.640	0.837	0.405−1.730			
NDUFS4[a]	inverse variance weighted	18	0.023	0.810	0.675−0.971	0.251	0.196	22.274
	MR Egger		0.430	0.901	0.701−1.159			
	simple mode		0.870	1.055	0.562−1.978			
	weighted mode		0.082	0.846	0.709−1.010			
MnSOD[a]	inverse variance weighted	13	0.043	1.262	1.008−1.581	0.276	0.899	22.269
	MR Egger		0.885	1.032	0.684−1.557			
	simple mode		0.920	1.031	0.571−1.862			
	weighted mode		0.112	1.264	0.967−1.653			
CUP3[a]	inverse variance weighted	19	0.005	0.634	0.462−0.869	0.899	0.293	23.430
	MR Egger		0.295	0.662	0.314−1.398			
	simple mode		0.559	0.773	0.330−1.806			
	weighted mode		0.660	0.829	0.365−1.883			

[a]GrpE1, GrpE protein homologue 1; HTRA2, serine protease HTRA2; ISCU, iron−sulfur cluster assembly enzyme; MCE, methylmalonyl-CoA epimerase; NDUFS4, NADH dehydrogenase iron−sulfur protein 4; MnSOD, superoxide dismutase [Mn]; CUP3, calcium uptake protein 3.

underpinnings of this association, thereby offering valuable insights to the medical community and fostering growth in this domain.

MATERIALS AND METHODS

Study Design. The MR study was predicated on three core assumptions, as depicted in Figure 1. The first assumption is that genetic instrumental variants exhibit a robust association with the exposure. The second assumption is that these genetic instrumental variants remain unaffiliated with any conceivable confounders. The third assumption is that such genetic instrumental variants correlate with the outcome solely through the conduit of exposure.[14,15]

Data Source. Data regarding mitochondrial-related exposure were gathered from IEUOpenGWAS. IEU OpenGWAS is an open-access database for genome-wide association studies

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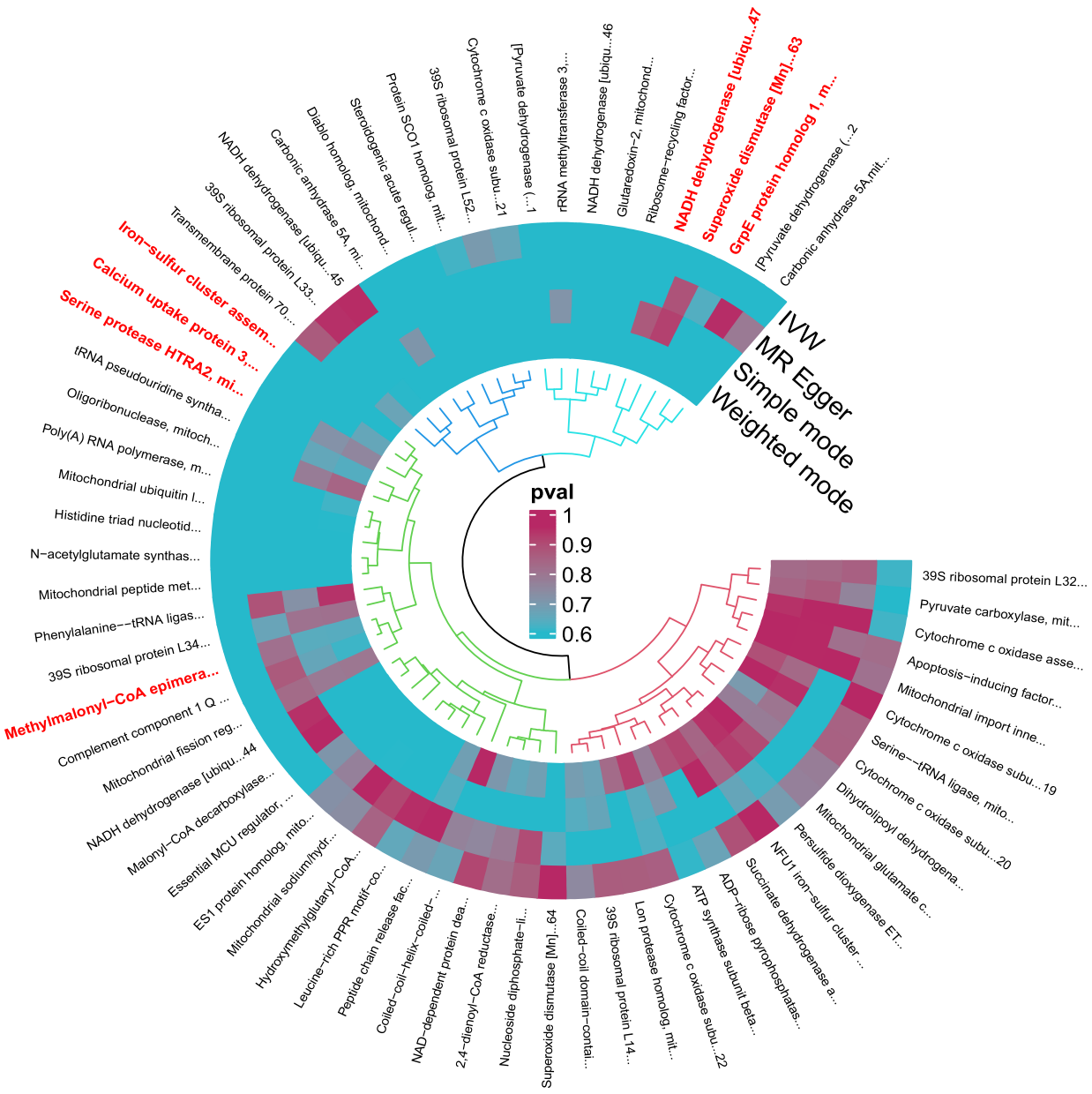

Figure 2. Circular analysis of mitochondrial functional proteins and sepsis association: significant proteins highlighted.

aimed at promoting genetic epidemiology research. To identify gene loci associated with these metabolites, single-nucleotide polymorphisms (SNPs) that met suggestive genome-wide significance thresholds ($P < 5 \times 10^{-5}$) were used as IVs (Table 1).[16]

The GWAS summary statistics for sepsis were sourced from the UK Biobank consortium and can also be accessed through the IEUOpenGWAS Web site, under the identifiers ieu-*b*-4982. The data set comprises a sample size of 431,365, of which 1380 individuals were diagnosed with sepsis and 347 experienced mortality within a 28-day period.

Instrumental Variable Selection. The following criteria were employed to select the IVs: (1) Single-nucleotide polymorphisms associated with each genus at the locus-wide significance threshold ($P < 1 \times 10^{-5}$) were selected as potential IVs; (2) 1000 Genomes Project European samples data were used as the reference panel to calculate the linkage disequilibrium between the SNPs, and among those SNPs that had $r^2 < 0.001$ (clumping window size = 10,000 kb), only the SNPs with the lowest P values were retained; (3) SNPs with minor allele frequency ≤0.01 were removed; and (4) when palindromic SNPs existed, the forward strand alleles were inferred using allele frequency information.[17]

The strength of IVs was assessed by calculating the F-statistic using the formula $F = R^2 \times (N - 1 - K)/((1 - R^2) \times K)$, where R^2 represents the proportion of variance in the exposure explained by the genetic variants, N represents the sample size, and K represents the number of instruments. When the corresponding F-statistic was >10, it was considered that there was no significant weak instrumental bias.[18]

Mendelian Randomization Analysis. We employed four methods for conducting MR analysis, including the inverse variance weighted (IVW), multiplicative random effects (MR-Egger), simple mode, and weighted mode. The IVW method was primarily used due to its high statistical efficiency and common application when all SNPs are considered valid

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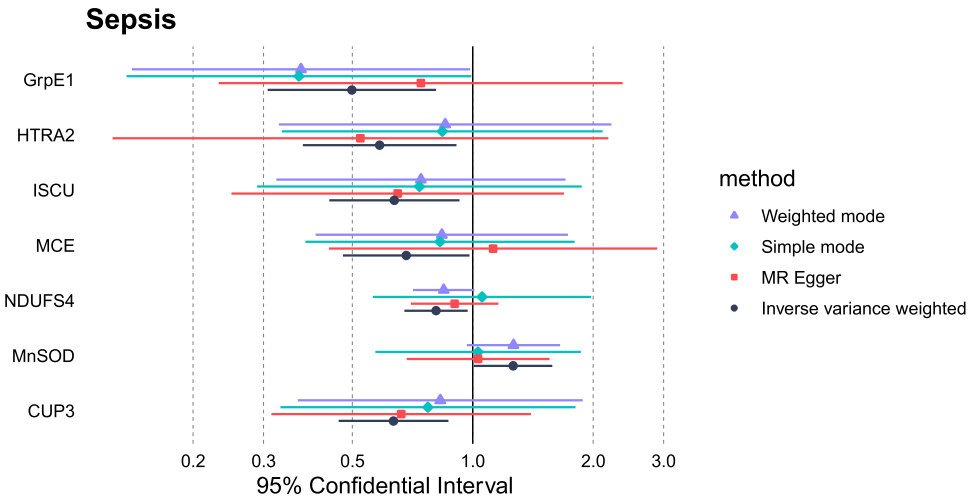

Figure 3. Forest plot of Mendelian randomization analysis on the impact of mitochondrial-related proteins on sepsis. GrpE1, GrpE protein homologue 1; HTRA2, serine protease HTRA2; ISCU, iron−sulfur cluster assembly enzyme; MCE, methylmalonyl-CoA epimerase; NDUFS4, NADH dehydrogenase iron−sulfur protein 4; MnSOD, superoxide dismutase [Mn]; CUP3, calcium uptake protein 3.

instruments, ideal for large samples but sensitive to pleiotropic biases. MR-Egger regression was implemented as a robust alternative, especially useful in addressing horizontal pleiotropy by providing bias-adjusted estimates, albeit with generally lower power and stricter model assumptions. Simple mode and weighted mode methods were also utilized, with simple mode offering straightforward calculations and ease of understanding for consistent effect sizes, yet it is sensitive to outliers. Weighted mode enhances estimate stability by adjusting for individual SNP influence, particularly when there are significant variations in effect sizes among SNPs, although it requires careful weight allocation.[19−21] Sensitivity analyses were conducted to assess the robustness of our findings, particularly focusing on the detection and address of horizontal pleiotropy. To this end, the MR-Egger regression was employed as the sole method for its recognized ability in providing insights into potential pleiotropic effects. Furthermore, Cochran's Q test was employed to evaluate the heterogeneity between SNPs associated with each microbial taxon.[22] Lastly, a leave-one-out sensitivity analysis was conducted to evaluate the influence of individual SNPs on the overall estimates. All MR analyses were performed in R (version 4.1.2) using the TwoSampleMR package.[23]

RESULT

Utilizing the two-sample Mendelian Randomization approach, we examined data from the IEUOpenGWAS database, encompassing 66 exposures related to mitochondrial functionality as well as sepsis data from the UKbiobank database. Four distinct Mendelian randomization methodologies were employed: IVW, MR Egger, simple mode, and weighted mode to evaluate the potential causal interplay between exposures and sepsis. Among these 66 mitochondrial-associated exposures, the IVW analytical method yielded significant results for 7 proteins, namely, GrpE protein homologue 1(GrpE1), serine protease HTRA2 (HTRA2), iron−sulfur cluster assembly enzyme (ISCU), methylmalonyl-CoA epimerase (MCE), NADH dehydrogenase iron−sulfur protein 4 (NDUFS4), superoxide dismutase [Mn] (MnSOD), and calcium uptake protein 3(CUP3), as shown in Figure 2, seemingly indicative of a connection to the onset of sepsis in ICU. All IV F-values exceeded 10, with no bias from weak instruments detected (Figure 3).

GrpE1and Sepsis. Using the IVW method, a significant association was found between GrpE1 and sepsis, with a P value of 0.005 and an OR of 0.499 (95% CI: 0.307−0.810). Since the OR is less than 1, this suggests that GrpE1 might have a protective effect, reducing the risk of sepsis. In all MR analyses, GrpE1 consistently demonstrated this protective effect, with no significant heterogeneity (P = 0.636) or pleiotropy (P = 0.495) observed.

ISCU and Sepsis. The IVW method indicated a significant association between ISCU and sepsis, with a value of 0.018 and an OR of 0.637 (95% CI: 0.438−0.926). Similarly, given that the OR is less than 1, this suggests that ISCU might have a protective effect, reducing the risk of sepsis. No significant heterogeneity (P = 0.379) or pleiotropy (P = 0.966) was observed.

HTRA2 and Sepsis. IVW analysis showed a significant association between HTRA2 and sepsis, with a P value of 0.018 and an OR of 0.585 (95% CI: 0.376−0.911). As the OR is less than 1, this indicates that HTRA2 might have a protective effect, reducing the risk of sepsis. No significant heterogeneity (P = 0.374) or pleiotropy (P = 0.877) was observed.

CUP3 and Sepsis. Using the IVW method, a significant association was found between CUP3 and sepsis, with a P value of 0.005 and an OR of 0.634 (95% CI: 0.462−0.869). No significant heterogeneity (P = 0.293) or pleiotropy (P = 0.899) was observed. Given that the OR is less than 1, this suggests that CUP3 might have a protective effect, reducing the risk of sepsis.

Relationship of Other Proteins with Sepsis. Proteins such as MCE, NDUFS4, and MnSOD all showed P values less than 0.05 in the IVW method but had P values greater than 0.05 in the other methods. Some of these proteins also exhibited heterogeneity and/or pleiotropy, suggesting that these associations might be incidental findings, and more research is needed for further validation.

Our results reveal a potential causal relationship between GrpE1, ISCU, HTRA2, and CUP3 and sepsis. For other proteins, despite some observed associations, further studies are required to validate these preliminary findings and explore their biological significance and potential mechanisms.

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DISCUSSION

In our investigation employing Mendelian randomization, we discerned that GrpE1, HTRA2, ISCU, and CUP3 are significantly associated with a reduced susceptibility to sepsis. The protective attributes of GrpE1, HTRA2, ISCU, and CUP3 align with their pivotal roles in mitochondrial function and cellular vitality, suggesting their potential contribution to mitigating sepsis risks, especially within the ICU milieu. Unearthing these potential protective proteins paves the way for targeted therapeutic interventions and pharmaceutical advancements against sepsis susceptibility.

GrpE1 exhibits multifaceted roles within the mitochondria. It predominantly collaborates with other heat-shock proteins, such as DnaK and DnaJ, ensuring proteins within the mitochondria retain their proper conformation.[13,24,25] Moreover, under stress conditions, particularly distress, GrpE expression escalates, aiding cells in preserving protein function against adversities.[26] This mechanism fortifies mitochondrial integrity, shielding them from deleterious effects. Animal studies substantiate that GrpE's function can fortify mice against bacterial infections.[27]Mitochondrial damage and dysfunction are intricately linked to sepsis progression. Given GrpE's capabilities, it potentially serves as a linchpin in safeguarding mitochondrial health, especially amidst a septic backdrop. Our findings bolster this perspective, positing GrpE1 as a protective element against sepsis onset. HTRA2, a mitochondrial protein, is renowned for its crucial role in cellular apoptosis and quality control. Its activity likely correlates with mitochondrial functionality and structural integrity, wherein mitochondrial functional impairment is perceived as a critical mechanism underlying sepsis and multiple organ dysfunction syndrome. Thus, our discoveries might shed light on the biological underpinnings of sepsis and potential therapeutic targets. ISCU, pivotal within the mitochondria, especially in iron−sulfur cluster biosynthesis, has emerged as a cornerstone auxiliary factor in the mitochondrial electron transport chain and various other biochemical pathways.[28−30] Due to mitochondria's cardinal role in cellular energy metabolism and stress response, the proper synthesis and functioning of iron−sulfur clusters are paramount for cellular vitality, offering protection against diverse infectious challenges.[31−33] Correspondingly, our research reveals that ISCU might harbor a protective association with sepsis onset. Preserving the iron−sulfur cluster's natural biosynthesis and functionality, especially in infectious and inflammatory contexts, could be instrumental in averting mitochondrial functional deficits and attenuating sepsis severity. CUP3 might regulate calcium ion uptake within cells, notably within mitochondria.[34] Calcium ions play an instrumental role in myriad biochemical and signal transduction pathways.[35,36]Mitochondrial calcium levels influence ATP production, enzymatic activities, and, in certain instances, cellular death pathways.[37,38] Within the context of sepsis, impaired cellular stress responses and mitochondrial functions are well-established. CUP3 could be crucial in maintaining mitochondrial calcium homeostasis, thus safeguarding mitochondrial functionality. Our research corroborates this view, suggesting that CUP3 might manifest protective effects against sepsis. Although we observed associations of several other proteins with sepsis, their associations were not stable in some MR methods. This may be attributed to statistical biases, confounders, or the choice of MR methods.

Mendelian randomization helps overcome confounding in observational studies and aims to provide accurate estimates. However, it has limitations. Pleiotropy, where genetic variants may affect outcomes outside the intended pathway, is addressed through methods such as MR-Egger regression, but these methods have their own limitations. Population stratification due to allele frequency and environmental differences across populations can also affect results, and while we have controlled for these factors, some residual confounding may remain. Additionally, the choice of statistical methods, including IVW and MR-Egger, influences the results; each has its own set of strengths and weaknesses.

Based on our findings, we propose focused suggestions for future research to enhance the understanding and treatment of sepsis and related ICU conditions. We will prioritize targeted therapeutic development, intensively explore drug repurposing with a particular focus on the proteins identified in our study, and conduct comprehensive in vivo and in vitro validation experiments. We recognize the importance of longitudinal and diverse population studies to ensure the broad applicability of our findings and will include these in future research. Additionally, we aim to extend our research to a wider array of ICU conditions beyond sepsis to further understand the disease dynamics and develop more effective treatments.

CONCLUSIONS

Our study has revealed that GrpE1, HTRA2, ISCU, and CUP3 might be related to the pathogenesis of sepsis. Further research is necessary to confirm and expand our findings, which could provide new strategies for the treatment and prevention of sepsis.

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Notes

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