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RESEARCH ARTICLE

Mendelian Randomization Study of the Relationship Between Serum Matrix Metalloproteinases and the Occurrence of Sepsis

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Abstract

Background Observational studies have shown that matrix metalloproteinases (MMPs) are associated with sepsis. However, it is unknown whether this association represents a causal relationship.

Methods Mendelian randomization (MR) analysis was conducted to assess the potential causal role of circulating MMPs in sepsis. Single nucleotide polymorphisms (SNPs) associated with circulating MMPs levels were used as instrumental variables (IVs). In a sepsis genome-wide association study comprising 1573 cases and 454,775 European ancestry controls, we examined these IVs' effects using a two-sample MR study. Causal estimates were calculated using inverse variance weighting (IVW), the weighted median method, and MR-Egger analysis.

Results Genetically predict that MMP-1 (OR = 1.011, 95% CI 0.772–1.325, $p = 0.936$), MMP-3 (OR = 1.036, 95% CI 0.862–1.244, $p = 0.707$), MMP-7 (OR = 1.206, 95% CI 0.960–1.515, $p = 0.108$), MMP-8 (OR = 1.041, 95% CI 0.949–1.144, $p = 0.395$), MMP-9 (OR = 1.101, 95% CI 0.831–1.458, $p = 0.503$), MMP-10 (OR = 1.028, 95% CI 0.840–1.260, $p = 0.789$) was not associated with the risk of sepsis.

Conclusions The MR study does not provide evidence that circulating levels of MMPs (1, 3, 7, 8, 9, 10) were the causes of sepsis.

Keywords Sepsis · Metalloproteinases (MMPs) · Mendelian randomization (MR) · Single nucleotide polymorphisms (SNPs)

1 Introduction

Sepsis is a condition where the host's dysregulated response to infection leading to life-threatening organ dysfunction [1]. Sepsis is a significant global health concern, with approximately 50 million cases occurring each year, and is the first cause of death among critically ill patients [2]. Matrix metalloproteinases (MMPs) are enzymes that require zinc to function and are involved in degrading and reactivating the extracellular matrix. The biological processes facilitated by MMPs include cell proliferation, differentiation, adhesion, angiogenesis, apoptosis, and inflammation [3, 4].

Studies on MMPs in sepsis have been extensively published [5]. Sepsis patients have higher circulating levels of MMP-1 and a decreased survival rate [6]. It is found that humans and mice deficient in both MMP-8 and tumor necrosis factor receptor 1 were more likely to survive in sepsis, and plasma MMP-8 was positively correlated with sequential organ failure assessment scores and interleukin-6(IL-6) [7]. Some observational studies have shown that MMP-9 is upregulated during septic shock, thereby causing thrombocytopenia [8] and reduced MMP-9/tissue inhibitor of metalloproteinase-1(TIMP-1) ratio may lead to multi-organ dysfunction [9]. The results of these studies confirm the importance of MMPs in sepsis, considering them as promising biomarkers for diagnosis.

Furthermore, such correlative evidence indicates that MMPs are associated with the risk of developing sepsis, but the causality cannot be established from observational studies. In light of the lack of randomized clinical trials (RCTs), to objectively test whether MMPs play a role in the development of sepsis, Mendelian randomization (MR)

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methods are employed to test causality. In MR, the effect of exposure (MMPs) on sepsis risk is examined using a genetic instrumental variable [10]. Because the instrumental variable is randomly distributed at conception, it is not subject to confounding effects. A common genetic variant associated with MMPs in genome-wide association studies (GWASs) serves as an instrumental variable for measuring MMPs. We explored the causal risk of developing sepsis from a genetic perspective to providing a basis for further prevention and diagnosis.

2 Methods

2.1 Study Design

Two-sample MR was conducted using GWAS data. The MR approach was based on the following three assumptions [11] (Fig. 1): Assumption 1, the genetic variant selected as instrumental variable is associated with MMPs; Assumption 2, the genetic variant is not associated with any unmeasured confounders; Assumption 3, the genetic variant is associated with sepsis only through MMPs, not through other pathways.

2.2 Data Sources

Association data for MMPs is compiled from the GWAS Catalog (https://www.ebi.ac.uk/gwas/). Recently, a large-scale proteogenomic study of 5368 Europeans indicated genetic variants related to 1989 serum proteins and common diseases were associated with 4035 independent associations between 2091 serum proteins, 36% of which had never been reported before [12]. The GWAS data for MMP-8 (n = 5365) and MMP-9 (n = 5357) were selected as a source of exposure data. Furthermore, in the recent large-scale mapping of protein quantitative trait loci, circulating levels of MMPs were measured among 90 candidate biomarkers related to cardiovascular risk, including MMP-1 (n = 16,889), MMP-3 (n = 20,791), MMP-7 (n = 18,245), and MMP-10 (n = 16,933) [13]. Detailed links to the corresponding data sources are provided (Supplementary Table S1).

Likewise, the genetic susceptibility to sepsis was obtained from the GWAS Catalog (https://www.ebi.ac.uk/gwas/) A GLMM-based GWA tool, fastGWA-GLMM, was applied to the UKB data of 456,348 individuals, 11,842,647 variants, and 2989 binary traits and identified 259 rare variants associated with 75 traits, illustrating the use of imputed genotype data in a large cohort to identify rare variants for binary complex traits [14]. Sepsis cases were collected from the UK Biobank, which included 1573 sepsis cases, defined as explicit sepsis [2], and 454,775 control subjects. Data from sepsis are selected from the final summary data, and links to sources are provided (Supplementary Table S1). Since all data were derived from previously published GWASs, the study did not require ethical approval.

2.3 Genetic Variants

As for the exposure data, genetic variants with genome-wide association thresholds ($p < 5.0 \times 10^{-6}$) were selected as instrumental variables (IVs) and applied a clumping algorithm (r2 threshold = 0.001; kb = 10 mB) to eliminate linkage disequilibrium. Additionally, strongly correlated IVs were selected based on F-statistics > 10 (F-value calculation formula below) to satisfy the correlation assumption of MR analysis: variants are strongly correlated with exposure [15].

$$F = \frac{Beta^2}{Se^2}$$

Beta is the effect size of the single-nucleotide polymorphisms (SNPs) of exposure; SE is the standard error of the SNP of exposure)

It was found that 7 SNPs were associated with MMP-1, 7 SNPs were associated with MMP-3, 4 SNPs were associated with MMP-7, 7 SNPs were associated with MMP-8, 3 SNPs were associated with MMP-9, and 6 SNPs were associated with MMP-10. The effect size was measured in the MMPs change of circulating concentration for each additional effect allele, as shown in Supplementary Table S2-7. No linkage disequilibrium was found between SNPs.

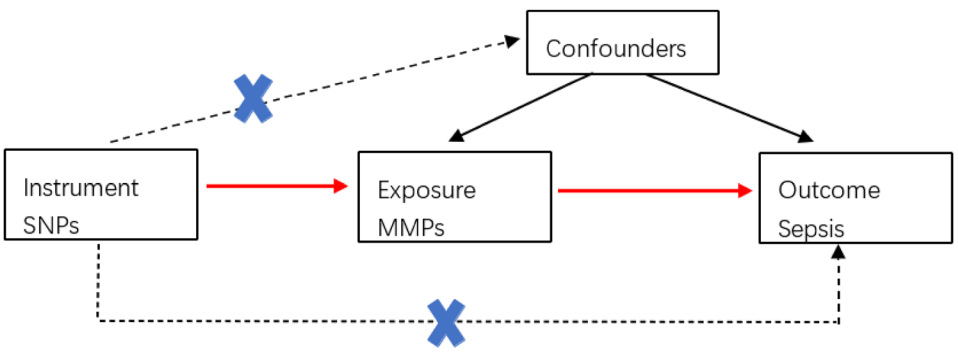

Fig. 1 The theory of Mendelian randomization analysis. The broken lines indicate potential pleiotropic or direct causal effects between variables that would not be consistent with the Mendelian randomization assumptions. *SNP* single-nucleotide polymorphism, *MMP* matrix metalloproteinase

2.4 Statistical Analysis

Mendelian analysis was primarily performed using inverse variance weighting (IVW), which provides a combined causal estimate from each SNP. IVW is considered conventional MR since it is equivalent to a two-stage least squares or allele score analysis using individual-level data [16]. However, horizontal polymorphism and null IV can lead to biased IVW estimates, so two complementary methods were applied. The Weighted Median method will provide reliable effect estimates with less than half of the IVs invalid. Mendelian randomization-Egger (MR-Egger) analysis was used to evaluate the pleiotropy effects [11]. Similarly, sensitivity analyses were conducted to determine reliable results [17]. The results of MMPs and sepsis were first tested for heterogeneity and horizontal multiplicity, followed by a leave-one-out analysis computed the meta-effect of remaining SNPs through iterative culling of each SNP. Leave-one-out analysis demonstrated the robustness of the causal relationship between exposure and outcome.

The statistical analysis was performed using R software (version 4.2.1) and the "TwoSampleMR" package in Rstudio (version 0.5.6), and the overall estimates of IVW, MR-Egger, and Weighted median methods were performed using the "forestploter" package (version 0.2.6). A Bonferroni-corrected significance threshold of $p = 0.05/6$, but we prespecified a Bonferroni-corrected significance threshold of $p = 0.008$ to adjust for multiple testing with a view to six exposures. Associations with p values between 0.05 and 0.008 were considered suggestive evidence of a possible association.

3 Results

In the primary analysis, IVW was used to provide estimates of the causal relationship between MMPs and sepsis, indicating that MMP-1 (OR = 1.011, 95% CI 0.772–1.325, $p = 0.936$), MMP-3 (OR = 1.036, 95% CI 0.862–1.244, $p = 0.707$), MMP-7 (OR = 1.206, 95% CI 0.960–1.515, $p = 0.108$), MMP-8 (OR = 1.041, 95% CI 0.949–1.144, $p = 0.395$), MMP-9 (OR = 1.101, 95% CI 0.831–1.458, $p = 0.503$), MMP-10 (OR = 1.028, 95% CI 0.840–1.260, $p = 0.789$) were not significantly associated with the risk of sepsis.

MR-Egger's and the weighted median method's overall estimates were consistent with the IVW analysis (Table 1). Moreover, the sensitivity analysis revealed no horizontal pleiotropy or heterogeneity (Supplementary Table S8). After systematically eliminating SNP through the leave-one-out analysis, the outcomes show minimal alteration, demonstrating that no single variant influenced the association between MMPs (1, 3, 7, 8, 9, 10) and sepsis (Fig. 2).

Table 1 Mendelian randomization results for causal effects of matrix metalloproteinases on sepsis

Trait	SNPs	IVW		MR-Egger		WM		Heterogeneity		Pleiotropy	
		OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	I^2	P	Egger intercept	P
MMP-1	7	1.011 (0.772–1.325)	0.936	0.828 (0.506–1.357)	0.488	0.993 (0.827–1.193)	0.941	59.91%	0.021	0.054	0.386
MMP-3	7	1.036 (0.862–1.244)	0.707	0.945 (0.689–1.296)	0.740	1.049 (0.853–1.290)	0.651	0	0.856	0.022	0.516
MMP-7	4	1.206 (0.960–1.515)	0.108	1.207 (0.849–1.717)	0.405	1.244 (0.974–1.588)	0.081	0	0.485	0	0.993
MMP-8	7	1.041 (0.949–1.144)	0.395	1.025 (0.883–1.190)	0.759	1.017 (0.933–1.110)	0.699	24.13%	0.245	0.014	0.788
MMP-9	3	1.101 (0.831–1.458)	0.503	1.083 (0.630–1.862)	0.822	1.114 (0.821–1.511)	0.489	0	0.855	0.005	0.955
MMP-10	6	1.028 (0.840–1.260)	0.789	1.032 (0.752–1.418)	0.853	1.027 (0.850–1.242)	0.780	36.82%	0.161	-0.002	0.972

Estimates of Inverse-variance weighted, MR-Egger, and Weighted median of matrix metalloproteinases on sepsis in the Mendelian randomization analysis

MMP matrix metalloproteinases, *SNPs* single nucleotide polymorphisms, *IVW* inverse variance weighted, *WM* weighted median, *OR* odds ratio, *CI* confidence interval, *MR-Egger* Mendelian randomization-Egger

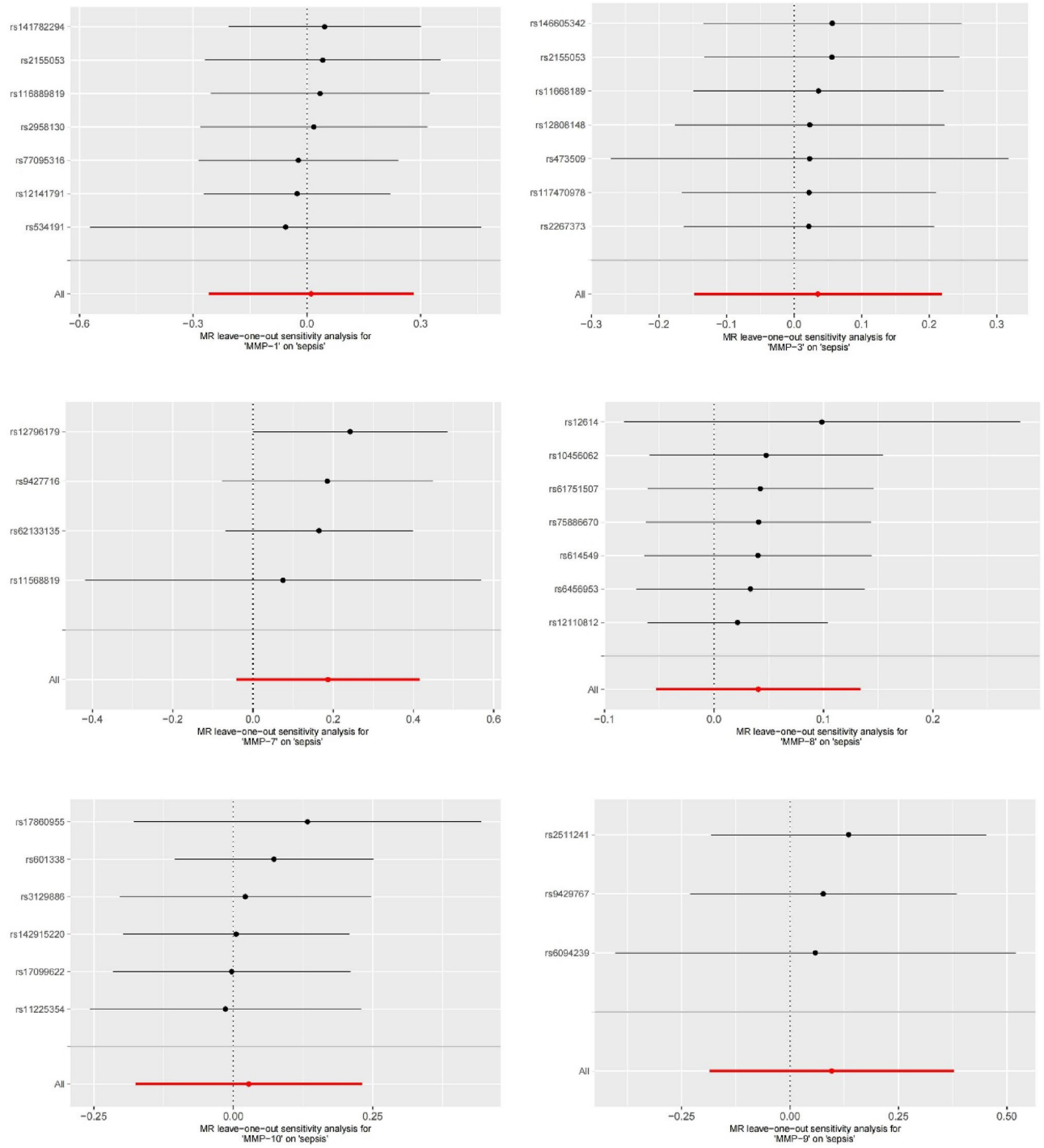

Fig. 2 Leave-one-out analysis of matrix metalloproteinases on sepsis in the Mendelian randomization study. *MR* Mendelian randomization, *MMPs* matrix metalloproteinases

4 Discussion

Genetic variants are used to assess whether a risk factor has a causal impact on an outcome in MR study. As far as we are aware, this is the first MR study to evaluate the effects of MMPs on the occurrence of sepsis. Consequently, the study found no causal relationship between MMPs (1, 3, 7, 8, 9, 10) and sepsis in Europeans. In other words, altered levels of circulating MMPs are unrelated to the development of sepsis.

MMPs could degrade all components of the extracellular matrix, thus facilitating cell migration within tissues and increasing the availability of growth factors bound to the matrix. Previous observational studies identified that elevated plasma levels of MMP-1, MMP-3, MMP-8, MMP-9, and MMP-10 were linked with the severity of sepsis and mortality in sepsis patients [18–23]. A prospective study noted that MMP-8 had an area under the ROC curve of 0.87 (95% CI 0.82–0.92) for the diagnosis of sepsis. MMP-9 had an area under the receiver operating characteristic (ROC) curve of 0.73 (95% CI 0.65–0.80) for detection of a non-septic state, and sepsis is associated with a higher plasma level of MMP-8 and MMP-9 [24]. Animal studies of sepsis discovered that reducing or inhibiting MMP-9 and MMP-8 was associated with improved survival and outcomes. MMP8 has therefore been considered a promising biomarker for treating sepsis [7, 25]. Therefore, MMPs have been widely recognized and studied as potential contributors to sepsis during the past decade. However, our results do not prove a causal relationship between MMPs and sepsis risk.

In addition, MMPs and sepsis have shown contradictory findings in published observational studies. Some studies showed that the circulating MMPs and TIMPs levels were not related to the risk of sepsis [24, 26] Furthermore, lipopolysaccharide administration causes the release of MMP-9 [27], and MMP-9 improves survival in patients with sepsis by mobilizing the bone marrow-derived endothelial progenitor cells that appear to assist in the neovascularization and endothelial repair of ischemic tissues [28, 29]. These studies support our findings that MMPs did not promote the occurrence of sepsis.

Several limitations have been identified in our study. First, we examined only a linear association between circulating MMPs (1, 3, 7, 8, 9, 10) and sepsis risk in the current MR study and did not find any evidence of a U-shaped relationship. Second, our study only examined the role of circulating MMPs and did not consider the role of intracellular MMPs in sepsis. Third, all the associated data for MMPs and sepsis were drawn from Europeans, so it is essential to exercise caution when applying these findings to Asian populations.

5 Conclusion

We did not find evidence of a causal relationship between circulating MMPs and sepsis to bolster the results of most previous observational studies. Previous studies have suggested that sepsis and MMPs may be connected, but confounding factors or reverse causality may also be at play. Sepsis and circulating MMPs require further research.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s44231-023-00047-2.

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Author Contributions CZY contributed to the conceptualization and methodology of the study. HYB and DHH played a part in the data analysis and visualization of results. QFZ and CL contributed to the validation of results and data curation. CZY and XL participated in drafting and reviewing the main manuscript. All authors reviewed and approved the manuscript.

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Data Availability Statement The original contributions presented in the study are included in the article/Supplementary Material. If you have any more questions, please feel free to contact the corresponding author for further information.

Declarations

Conflict of Interest The authors have no competing interests to declare relevant to this article's content.

Consent for Publication All individuals have individual rights that are not to be infringed. Individual participants in studies have, for example, the right to decide what happens to the (identifiable) personal data gathered, to what they have said during a study or an interview, as well as to any photograph that was taken. This is especially true concerning images of vulnerable people (e.g. minors, patients, refugees, etc.) or the use of images in sensitive contexts. In many instances authors will need to secure written consent before including images.

Ethics Approval Two-sample MR was conducted using GWAS data. The study did not require ethical approval since all data were derived from summary statistics from previously published GWASs.

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