Title Page

Article title

Causal effect of serum matrix metalloproteinase levels on venous thromboembolism: a Mendelian randomization study

Author information

Deheng Han

Department of Cardiology, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, 310003, China. E-mail address: 12118424@zju.edu.cn

Fangcong Yu

Department of Cardiology, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, 310003, China. E-mail address: 12318406@zju.edu.cn

Liangrong Zheng

Department of Cardiology, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, 310003, China. E-mail address: 1191066@zju.edu.cn

Corresponding author

Liangrong Zheng

Degree: MD, PhD. Telephone and fax number: +86 138 0574 9450

Department and affiliation: Department of Cardiology, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China.

Full postal address: No. 79 Qingchun Road, Hangzhou, Zhejiang Province, China

Short running head: Matrix metalloproteinase and venous thromboembolism

Conflicts of interest: none

Funding source: none

Causal effect of serum matrix metalloproteinase levels on venous thromboembolism: a Mendelian randomization study

ABSTRACT

Objectives

Serum matrix metalloproteinase (MMP) levels are associated with cardiovascular diseases. However, the causal associations between serum levels of specific MMPs and venous thromboembolism (VTE) remain unclear. The present study sought to explore the causal relationship between serum MMP levels and VTE by using the Mendelian randomization (MR) method.

Methods

In this study 2-sample MR study, the exposure data on serum MMP levels were derived from genome-wide association studies involving 21,758 individuals from 13 cohorts of European descent. The outcome data on VTE, including deep vein thrombosis and pulmonary embolism, were derived from the FinnGen research project. The primary method used was the inverse-variance weighting method. The MR-Egger intercept test and the Cochran Q test were used to evaluate pleiotropy and heterogeneity.

Results

Using the inverse-variance weighting method, higher serum MMP-12 levels were found to be associated with an increased risk of VTE (odds ratio, 1.04; 95% confidence interval, 1.01–1.07; p=0.0015). Moreover, there was a weak association between the levels of certain MMPs and

VTE. Sensitivity analyses revealed no significant heterogeneity and pleiotropy in our study, and the Steiger directionality test did not reveal a significant reverse causation association.

Conclusions

There is a causal association between MMP-12 levels and VTE, which may have substantial implications for the diagnostic and therapeutic strategies used for VTE.

Keywords: Venous thromboembolism, Mendelian randomization method, serum matrix metalloproteinases, deep vein thrombosis, pulmonary embolism

INTRODUCTION

Venous thromboembolism (VTE) is a multicausal disease that includes pulmonary embolism (PE) and deep vein thrombosis (DVT). It ranks as the third most common cardiovascular disease, affecting nearly 10 million people worldwide annually [1-3]. VTE is associated with both inherited genetic factors and acquired conditions, such as cancer, obesity, surgery, and infection [1] [4]. Understanding the pathophysiological mechanisms and risk factors associated with VTE is crucial for its effective prevention, diagnosis, and treatment.

Matrix metalloproteinases (MMPs) belong to the zinc-dependent endopeptidase family and have the ability to degrade virtually all constituents of the extracellular matrix, including elastin, fibronectin, and collagen, thereby promoting tissue repair and regeneration [5,6]. MMPs help maintain the delicate balance between extracellular matrix turnover and homeostasis under physiological conditions. Recent studies have revealed that MMPs can also participate in immune regulation, transcriptional control, and cell signaling [7]. Understanding the intricate biology of MMPs and their regulatory mechanisms has spurred advancements in basic,

preclinical, and clinical investigations. Clinical trials have particularly focused on the effectiveness of MMP inhibitors in treating a variety of diseases, including cardiovascular, neurological, oncological, and inflammatory conditions [8-11].

Mendelian randomization is a statistical method used to evaluate causal relationships by leveraging genetic variants as instrumental variables (IVs) [12]. Using genetic variants as IVs can prevent reverse causation and confounding bias, enabling a more accurate assessment of the causal relationship between exposures and outcomes [13-15].

To our knowledge, relatively few studies have investigated the relationship between MMP levels and VTE. The current study aimed to examine the causal effect of MMP levels on VTE (including PE and DVT). By elucidating the role of MMPs in VTE, we hope to identify novel diagnostic and therapeutic targets that can ultimately improve patient outcomes and reduce the burden of this life-threatening condition.

MATERIALS AND METHODS

Study design

This study utilized a 2-sample MR design to explore the causal associations between serum MMP levels and the occurrence of VTE (including PE and DVT). An MR study is based on 3 core assumptions. First, the instrumental variable is strongly associated with the exposure. Second, the instrumental variable is independent of confounders. Third, the instrumental variable affects the outcome solely via the exposure (Figure 1) [13-15].

Data source

The genetic variants associated with serum MMP levels were identified in a study by Folkersen et al. This research evaluated 90 candidate biomarkers linked to cardiovascular risk in 21,758 individuals across 1313 cohorts of European descent [16]. The statistical data are available for

download from the SCALLOP CVD-I online resource.

The FinnGen Project is a substantial public-private initiative that aims to collect and analyze genome and health data from 500,000 participants in Finnish biobanks. We extracted summary-level GWAS data for VTE (including DVT and PE) from the FinnGen consortium (Release 9, https://r9.finngen.fi/). This dataset included 19,372 cases and 357,905 controls for VTE (Phenocode: I9_VTE), 9,109 cases and 324,121 controls for DVT (Phenocode: I9_PHLETHROMBDVTLOW), and 9,243 cases and 367,108 controls for PE (Phenocode: I9_PULMEMB). The definitions of VTE, DVT, and PE were based on the International Classification of Diseases, ninth revision.

Selection of IVs

We selected the single nucleotide polymorphisms (SNPs) to be used as IVs based on the following criteria: First, SNPs were strongly associated with the MMP ($p<5\times10^{-8}$). Second, to prevent weak instrument bias, the F-statistic for each SNP was greater than 10. Third, LD clumping was used to exclude SNPs in linkage disequilibrium ($r^2<0.1$ and distance <10,000 kb) [17,18]. Fourth, the Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) test was used to remove potentially pleiotropic SNPs [19]. Fifth, to prevent reverse causality, Steiger filtering was used to identify SNPs indicative of causality in the reverse direction, which were then removed [20,21]. The detailed SNP statistics are presented in the supplemental Tables S2-S16. These include the SNP, sample size, effect allele, other allele, β, standard error, p-value, effect allele frequency (EAF), and the number of SNPs for the exposures and outcomes of all analyses.

Statistical analysis

The primary method used for this analysis was the random-effect inverse-variance weighting (IVW) method [20]. Complementary methods included the weighted mode, weighted median, MR-Egger, and simple mode methods. The MR-Egger intercept test and the Cochran Q test were utilized to assess horizontal pleiotropy and heterogeneity, respectively [22]. A p-value < 0.05 indicated the presence of horizontal pleiotropy and heterogeneity. We employed the MR-PRESSO method to identify heterogeneous outlier SNPs and to provide a corrected estimate after their removal [23,24].

To prevent reverse causality, Steiger filtering was employed to identify SNPs indicative of causality in the opposite direction. Additionally, the MR-Steiger directionality test was utilized in our analysis [21]. To mitigate the risk of weak instrument bias, the F-statistic for each SNP was calculated. R2 represents the variance explained by each SNP, calculated as $R^2 = 2 \times (1 - EAF) \times \beta^2 \times EAF$; $F = R^2 / (1 - R^2) \times (N - 2)$. Here, β represents the effect size; EAF denotes the effect allele frequency; and N is the number of individuals [25,26]. Power calculations were conducted using the online tool mRnd, based on the outcome sample size, proportion of cases, R^2 sum, and a type I error rate of 0.05 [27,28].

A scatter plot and leave-one-out plot were utilized to visualize the results of our study. Given the multiple analyses conducted, a Bonferroni-corrected p-value of less than 0.0033 (0.05/15) was deemed statistically significant. A p-value between 0.0033 and 0.05 was considered suggestive evidence. The analyses were performed using R software (version 4.2.3) and the TwoSampleMR R package.

RESULTS

Primary MR Analysis

Figure 2 presents the primary results of this MR study using the IVW method. A p-value less than 0.0033 was considered statistically significant, while a p-value ranging from 0.0033 to 0.05 was regarded as suggestive evidence. Seven trait pairs exhibited statistically significant differences before the Bonferroni correction was applied. Serum MMP-1 levels were associated with an increased risk of PE (odds ratio [OR], 1.06; 95% confidence interval [CI], 1.01–1.10; p=0.0104). Serum MMP-3 levels were associated with a higher risk of DVT (OR, 1.05; 95% CI, 1.01–1.09; p=0.0120) and a lower risk of PE (OR, 0.95; 95% CI, 0.92–0.99; p=0.0058). Serum MMP-7 levels were associated with a lower risk of PE (OR, 0.90; 95% CI, 0.84–0.97; p=0.0047). Serum MMP-10 levels were associated with a higher risk of VTE (OR, 1.04; 95% CI, 1.00–1.08; p=0.0461). Serum MMP-12 levels were associated with a higher risk of VTE (OR, 1.04; 95% CI, 1.01–1.07; p=0.0015) and PE (OR, 1.06; 95% CI, 1.02–1.10; p=0.0053). However, after applying the Bonferroni correction, only the association between serum MMP-12 levels and a higher risk of VTE remained significant. The scatter plots of significant MR results before the Bonferroni correction are displayed in Figure 3.

Sensitivity Tests

The weighted mode, MR-Egger, and weighted median methods were used to evaluate the causal association between MMP levels and VTE (including DVT and PE). Although several associations did not show statistical significance, the results were in the same direction as those obtained using the primary IVW method (Supplementary Table 1).

The pleiotropy of the study was evaluated using the MR-Egger regression method, and no significant pleiotropy was detected in our analyses (p for intercept > 0.05). The study's heterogeneity was assessed with the Cochran Q test, and no significant heterogeneity was observed in our analyses, except for the association between MMP-12 levels and PE (Table 1). Due to the presence of heterogeneity, both the MR-PRESSO outlier test and leave-one-out analysis were conducted to identify and eliminate outlier SNPs (Supplementary Figures S1-S7). Additionally, random-effect models were employed in our analysis to reduce the impact of heterogeneity. The MR-Steiger directionality test yielded a result of “true” for all tests, indicating the absence of reverse causal associations.

The statistical power for the main analyses met the 80% threshold, with the exception of the association between MMP-10 and VTE. This discrepancy may be attributed to the minimal variance in MMP-10 explained by the selected IVs. Therefore, caution is advised when interpreting this particular result. Overall, however, our results are considered relatively reliable.

DISCUSSION

In our study, we employed the MR method to explore the causal relationship between serum MMP levels and the incidence of VTE, which includes DVT and PE. Following Bonferroni correction, the findings suggested that elevated serum MMP-12 levels are associated with an increased risk of VTE.

To our knowledge, this study is the first to explore the causal relationship between serum MMP levels and VTE. VTE is a disease with multiple causes, including inherited genetic factors and acquired factors. It is also a common complication in patients who are bedridden for extended periods and those who have undergone surgery [1,4]. In clinical practice, D-dimer serves as a crucial laboratory marker for patients at low risk, boasting a sensitivity of up to 95% in

diagnosing VTE. However, its specificity is low [29]. Therefore, it is necessary to identify new biomarkers that offer both high sensitivity and specificity.

Some clinical and basic foundational studies have investigated the association between MMPs and thrombi. One study found that the upregulation of MMPs is involved in left atrial appendage thrombus formation in elderly people [30]. Another study demonstrated a disequilibrium of MMPs in the superficial venous wall in patients with superficial venous thrombosis. Therefore, MMPs may be implicated in the pathogenesis of superficial venous thrombosis [31]. Furthermore, in a mouse model of myocardial infarction, increased MMP levels were associated with the formation of intracardiac thrombi [32].

Previous studies also investigated the causal effect of MMP levels on ischemic stroke through Mendelian randomization. The first study examined the causal effects of MMP-1, MMP-8, and MMP-12 levels on ischemic stroke. It found that lower serum levels of MMP-12 were associated with an increased risk of ischemic stroke, lower serum levels of MMP-1 and MMP-12 were linked to an increased risk of large-artery stroke, and higher serum levels of MMP-8 were associated with an increased risk of small vessel stroke [33]. The second Mendelian study focused on the association between MMP-8 levels and ischemic stroke and its subtypes, finding that higher serum levels of MMP-8 were associated with increased risks of small vessel stroke [34]. The third Mendelian study investigated promising therapeutic targets for ischemic stroke identified from plasma and cerebrospinal fluid proteomes. Using different databases, it found that lower serum levels of MMP-12 were associated with an increased risk of ischemic stroke [35]. These 3 Mendelian randomization studies showed relatively consistent results.

However, epidemiological studies investigating the association between MMP-8 levels and the risk of stroke have yielded inconsistent results; several studies have demonstrated a significant correlation [36,37], while others have not [38,39]. There are many reasons for this divergence. First, observational studies suffer from several methodological limitations for causal inference,

192 including inherent biases such as confounding and reverse causation. Second, the databases for
193 stroke in Mendelian randomization studies were mainly from individuals of European descent,
194 whereas the observational cohort was ethnically diverse. Third, the clotting process during
195 serum preparation is known to release MMPs from circulating leukocytes. Therefore,
196 measuring this proteinase from serum also reflects the potential of the neutrophils to
197 degranulate and release it, and this degree may depend on genetic variations [40]. Overall,
198 larger studies are needed to confirm the causal relationships between serum MMP levels and
199 stroke.

200 Although the precise relationship between MMP-12 levels and VTE is not fully understood,
201 with ongoing research, blood stasis, endothelial damage, and hypercoagulability are recognized
202 as the three primary components of thrombosis [41]. MMP-12 was first identified and
203 characterized in macrophages [42,43]. This macrophage-derived MMP-12 can break down the
204 protein that connects endothelial cells, resulting in cell apoptosis and injury [43]. Additionally,
205 in a model of vascular injury, biomarkers associated with vascular damage were found to be
206 reduced in MMP-12 knockout mice [44].

207 In addition to its degradative mechanism, MMP-12 promotes inflammatory responses, which
208 are crucial for the development of thrombosis. In mice with MMP-12 overexpression, there is
209 an increase in chemokine secretion and the recruitment of inflammatory cells, such as
210 macrophages, to the sites of inflammation [45]. In MMP-12 knockout mice, the inflammation
211 was comparatively more attenuated than in control mice [46].

212 Furthermore, MMP-12 has been implicated in the regulation of fibrinolysis, a process in which
213 blood clots are broken down [47]. It inhibits fibrinolysis by degrading plasminogen activators,
214 which are essential for the breakdown of fibrin clots. This inhibition may contribute to the
215 persistence and growth of blood clots in VTE [48,49]. Overall, the relationship between MMP-

12 levels and VTE involves vascular injury, modulation of inflammatory responses, and regulation of fibrinolysis. However, further research is needed to fully understand the complex interactions and potential mechanisms involved in this association.

In our analysis, before applying the Bonferroni correction, serum MMP-3 levels were associated with an increased risk of DVT (OR, 1.05; 95% CI, 1.01–1.09; p=0.0120) and a decreased risk of PE (OR, 0.95; 95% CI, 0.92–0.99; p=0.0058). However, these associations disappeared after the Bonferroni correction was applied. Pulmonary embolism comprises a group of clinical syndromes characterized by the obstruction of the pulmonary artery or its branches by various emboli. These emboli can include materials such as thrombus, fat, air, amniotic fluid, bone marrow, metastatic cancer, bacteria, and cardiac organisms. Deep vein thrombosis involves the clotting of venous blood within the deep veins of the lower extremities and does not necessarily result in pulmonary embolism. Further research is required to elucidate these discrepancies.

All results presented in this study were based on the IVW method. Various types of sensitivity analysis further confirmed the strength and reliability of our findings. The IVW method is likely to provide the most reliable causal estimates. When exploring the association between MMP-12 levels and VTE, the MR Egger method (p=0.087) and the weighted mode method (p=0.006) appeared to be somewhat inconsistent with the main findings. Although the p-values did not reach statistical significance, the different MR methods (IVW, MR-Egger, and weighted mode) demonstrated directionally consistent results, leading us to consider our results robust.

An advantage of this study is that it employed the MR method to investigate the relationship between MMP levels and VTE, effectively reducing the influence of confounding factors and avoiding erroneous causal associations. The GWAS data for MMPs and VTE were obtained from a large cohort. Furthermore, sensitivity analyses showed no significant horizontal pleiotropy or heterogeneity in our study, and the Steiger directionality test did not indicate any significant reverse causal relationships.

However, our study also had some limitations. First, although the cohort was considerably large, it is important to note that all patients were of European descent. Therefore, the findings may not be applicable to other populations. Second, we investigated only the causal association between five specific MMPs and VTE. The GWAS data for other MMPs were relatively limited, resulting in an extremely small number of SNPs available for analysis. Third, we could not perform a subgroup analysis because the GWAS data consisted only of summary-level statistics. Fourth, the statistical power for the association between MMP-10 and VTE did not reach the 80% threshold, which could be due to the minimal variance in MMP-10 explained by the selected instrumental variables. Therefore, caution is advised when interpreting the results.

Conclusions

In conclusion, this MR study established a causal relationship between MMP-12 levels and VTE, which could significantly impact the diagnostic and therapeutic approaches for VTE. Further research is required to confirm these findings and investigate the underlying mechanisms.

Data availability

All the datasets used in the present study are publicly available. The data generated or analyzed during this study have been included in this published article.

Conflict of Interest

The authors have no conflicts of interest to declare for this study.

Funding

None

Acknowledgement

The genetic variants of the serum MMP levels were obtained from a study conducted by Folkersen et al., and the GWAS data of venous thromboembolism were obtained from the FinnGen research project. We thank the participants for sharing the data.

Author contributions

Conceptualization: Han D, Zheng L. Data curation: Han D Yu F. Formal analysis: Han D Yu F. Funding acquisition: None. Methodology: Han D. Project administration: Han D Yu F. Visualization: Han D Yu F. Writing – original draft: Han D Yu F. Writing – review & editing: Zheng L.

ORCID

REFERENCES

1. Khan F, Tritschler T, Kahn SR, Rodger MA. Venous thromboembolism. Lancet. 2021;398(10294):64-77.
2. Lutsey PL, Zakai NA. Epidemiology and prevention of venous thromboembolism. Nat Rev Cardiol. 2023;20(4):248-262.
3. Duffett L. Deep Venous Thrombosis. Ann Intern Med. 2022;175(9):ITC129-ITC144.
4. Guntupalli SR, Spinosa D, Wethington S, Eskander R, Khorana AA. Prevention of venous thromboembolism in patients with cancer. BMJ. 2023;381:e072715.
5. de Almeida LGN, Thode H, Eslambolchi Y, et al. Matrix Metalloproteinases: From Molecular Mechanisms to Physiology, Pathophysiology, and Pharmacology. Pharmacol Rev. 2022;74(3):712-768.
6. Mahalanobish S, Saha S, Dutta S, Sil PC. Matrix metalloproteinase: An upcoming therapeutic approach for idiopathic pulmonary fibrosis. Pharmacol Res. 2020;152:104591.
7. Li K, Tay FR, Yiu CKY. The past, present and future perspectives of matrix metalloproteinase inhibitors. Pharmacol Ther. 2020;207:107465.
8. Chen AY, Adamek RN, Dick BL, Credille CV, Morrison CN, Cohen SM. Targeting Metalloenzymes for Therapeutic Intervention [published correction appears in Chem Rev. 2019 Jun 11;:]. Chem Rev. 2019;119(2):1323-1455.
9. Bräuninger H, Krüger S, Bacmeister L, et al. Matrix metalloproteinases in coronary artery disease and myocardial infarction. Basic Res Cardiol. 2023;118(1):18.
10. Grillet B, Pereira RVS, Van Damme J, Abu El-Asrar A, Proost P, Opdenakker G. Matrix metalloproteinases in arthritis: towards precision medicine. Nat Rev Rheumatol.

2023;19(6):363-377.

11. Piperigkou Z, Karamanos NK. Estrogen receptor-mediated targeting of the extracellular matrix network in cancer. Semin Cancer Biol. 2020;62:116-124.

12. Li W, Zhang H, Ren A, et al. Systemic lupus erythematosus is associated with lower risk of hepatitis B virus infection: A multivariable Mendelian randomization study in East Asian population. J Med Virol. 2023;95(11):e29226.

13. Emdin CA, Khera AV, Kathiresan S. Mendelian randomization. JAMA 2017; 318 (19) 1925-1926.

14. Davies NM, Holmes MV, Davey Smith G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. BMJ 2018; 362: k601.

15. Lawlor DA. Commentary: Two-sample Mendelian randomization: opportunities and challenges. Int J Epidemiol 2016; 45 (03) 908-915.

16. Folkersen L, Gustafsson S, Wang Q, et al. Genomic and drug target evaluation of 90 cardiovascular proteins in 30,931 individuals. Nat Metab. 2020;2(10):1135-1148.

17. Tan JS, Liu NN, Guo TT, Hu S, Hua L. Genetically predicted obesity and risk of deep vein thrombosis. Thromb Res 2021; 207: 16-24.

18. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. Nat Genet 2018; 50 (05) 693-698 .

19. Bowden J, Spiller W, Del Greco M F. et al. Improving the visualization, interpretation and analysis of two-sample summary data Mendelian randomization via the Radial plot and Radial regression. Int J Epidemiol 2018; 47 (04) 1264-1278.

20. Palmer TM, Lawlor DA, Harbord RM, et al. Using multiple genetic variants as instrumental variables for modifiable risk factors. Stat Methods Med Res. 2012;21(3):223-242 .
21. Chen H, Chen S, Ye H, Guo X. Protective Effects of Circulating TIMP3 on Coronary Artery Disease and Myocardial Infarction: A Mendelian Randomization Study. J Cardiovasc Dev Dis. 2022;9(8):277. Published 2022 Aug 18.
22. Huedo-Medina TB, Sánchez-Meca J, Marín-Martínez F, Botella J. Assessing heterogeneity in meta-analysis: Q statistic or I2 index?. Psychol Methods 2006; 11 (02) 193-206.
23. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. Int J Epidemiol 2015; 44 (02) 512-525.
24. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. Nat Genet 2018; 50 (05) 693-698.
25. Liang X, Fan Y. Bidirectional two-sample Mendelian randomization analysis reveals a causal effect of interleukin-18 levels on postherpetic neuralgia risk. Front Immunol. 2023;14:1183378.
26. Zhao Z, Cao Q, Zhu M, Wang C, Lu X. Causal relationships between serum matrix metalloproteinases and estrogen receptor-negative breast cancer: a bidirectional mendelian randomization study. Sci Rep. 2023;13(1):7849.
27. Lee KJ, Kim H, Lee SJ, et al. Causal Effect of the 25-Hydroxyvitamin D Concentration on Cerebral Small Vessel Disease: A Mendelian Randomization Study. *Stroke*. 2023;54(9):2338-2346.

28. Dale CE, Fatemifar G, Palmer TM, et al. Causal Associations of Adiposity and Body Fat Distribution With Coronary Heart Disease, Stroke Subtypes, and Type 2 Diabetes Mellitus: A Mendelian Randomization Analysis. *Circulation*. 2017;135(24):2373-2388.
29. van Es N, Takada T, Kraaijpoel N, et al. Diagnostic management of acute pulmonary embolism: a prediction model based on a patient data meta-analysis. Eur Heart J. 2023;44(32):3073-3081.
30. Tan R, Yuan M, Wang L, et al. The pathogenesis of aging-induced left atrial appendage thrombus formation and cardioembolic stroke in mice is influenced by inflammation-derived matrix metalloproteinases. Thromb Res. 2023;226:69-81.
31. Yu G, Li K, Xu Y, Chu H, Zhan H, Zhong Y. The expression of matrix metalloproteinases and their tissue inhibitors in the vein wall following superficial venous thrombosis. Phlebology. 2022;37(1):63-71.
32. Kaireviciute D, Blann AD, Balakrishnan B, et al. Characterisation and validity of inflammatory biomarkers in the prediction of post-operative atrial fibrillation in coronary artery disease patients. Thromb Haemost. 2010;104(1):122-127.
33. Cárcel-Márquez J, Cullell N, Muiño E, et al. Causal Effect of MMP-1 (Matrix Metalloproteinase-1), MMP-8, and MMP-12 Levels on Ischemic Stroke: A Mendelian Randomization Study. Stroke. 2021;52(7):e316-e320.
34. Jia Y, Guo D, Zhang K, et al. Causal associations of serum matrix metalloproteinase-8 level with ischaemic stroke and ischaemic stroke subtypes: a Mendelian randomization study. *Eur J Neurol*. 2021;28(8):2543-2551.
35. Zou X, Wang L, Wang S, et al. Promising therapeutic targets for ischemic stroke identified

366 from plasma and cerebrospinal fluid proteomes: a multicenter Mendelian randomization
367 study. *Int J Surg*. 2024;110(2):766-776. Published 2024 Feb 1.
368 36. Palm F, Lahdentausta L, Sorsa T, et al. Biomarkers of periodontitis and inflammation in
369 ischemic stroke: a case–control study. Innate Immun. 2014; 20(5): 511-518.
370 37. Hsieh FI, Chiou HY, Hu CJ, et al. Combined effects of MMP-7, MMP-8 and MMP-26 on
371 the risk of ischemic stroke. J Clin Med. 2019; 8(11): 2011.
372 38. Peeters W, Moll FL, Vink A, et al. Collagenase matrix metalloproteinase-8 expressed in
373 atherosclerotic carotid plaques is associated with systemic cardiovascular outcome. *Eur*
374 *Heart J*. 2011;32(18):2314-2325.
375 39. Kormi I, Nieminen MT, Havulinna AS, et al. Matrix metalloproteinase-8 and tissue
376 inhibitor of matrix metalloproteinase-1 predict incident cardiovascular disease events and
377 all-cause mortality in a population-based cohort. *Eur J Prev Cardiol*. 2017;24(11):1136-
378 1144.
379 40. Tuomainen AM, Nyyssonen K, Laukkanen JAet al. Serum matrix metalloproteinase-8
380 concentrations are associated with cardiovascular outcome in men. Arterioscler Thromb
381 Vasc Biol.2007; 27: 2722–2728.
382 41. Kennedy R, Schneier A, Javed M, Truong H. Recurrent upper extremity arterial thrombosis
383 preceding a diagnosis of COVID-19. Ann Vasc Surg Brief Rep Innov. 2023;3(1):100148
384 42. Kerzeli IK, Kostakis A, Türker P, et al. Elevated levels of MMP12 sourced from
385 macrophages are associated with poor prognosis in urothelial bladder cancer. BMC Cancer.
386 2023;23(1):605.
387 43. Zhou X, Zhang C, Yang S, et al. Macrophage-derived MMP12 promotes fibrosis through

sustained damage to endothelial cells. J Hazard Mater. 2024;461:132733.

44. Stawski L, Haines P, Fine A, Rudnicka L, Trojanowska M. MMP-12 deficiency attenuates angiotensin II-induced vascular injury, M2 macrophage accumulation, and skin and heart fibrosis. PLoS One. 2014;9(10):e109763.

45. Aristorena M, Gallardo-Vara E, Vicen M, et al. MMP-12, Secreted by Pro-Inflammatory Macrophages, Targets Endoglin in Human Macrophages and Endothelial Cells. Int J Mol Sci. 2019;20(12):3107.

46. Amor M, Bianco V, Buerger M, et al. Genetic deletion of MMP12 ameliorates cardiometabolic disease by improving insulin sensitivity, systemic inflammation, and atherosclerotic features in mice. Cardiovasc Diabetol. 2023;22(1):327.

47. Belaaouaj AA, Li A, Wun TC, Welgus HG, Shapiro SD. Matrix metalloproteinases cleave tissue factor pathway inhibitor. Effects on coagulation. J Biol Chem. 2000;275(35):27123-27128.

48. Challa SR, Nalamolu KR, Fornal CA, et al. The interplay between MMP-12 and t-PA in the brain after ischemic stroke. Neurochem Int. 2022;161:105436.

49. Koolwijk P, Sidenius N, Peters E, et al. Proteolysis of the urokinase-type plasminogen activator receptor by metalloproteinase-12: implication for angiogenesis in fibrin matrices. Blood. 2001;97(10):3123-3131.

408

409 **Table 1.** Heterogeneity and pleiotropy tests of the significance of causal effects of MMPs on

410 VTE

Exposure	Outcome	Pleiotropy			Heterogeneity	
		Intercept	SE	p-value	Q	p-value
MMP1	VTE	-0.005	0.006	0.448	52.521	0.059
MMP1	DVT	0.007	0.009	0.469	54.215	0.053
MMP1	PE	0.004	0.009	0.658	52.579	0.072
MMP3	VTE	0.007	0.005	0.148	61.105	0.208
MMP3	DVT	0.001	0.007	0.841	62.543	0.199
MMP3	PE	-0.002	0.006	0.760	56.594	0.378
MMP7	VTE	-0.001	0.007	0.854	21.751	0.194
MMP7	DVT	0.000	0.009	0.988	11.071	0.853
MMP7	PE	-0.009	0.010	0.367	20.029	0.219
MMP10	VTE	0.003	0.007	0.682	38.413	0.072
MMP10	DVT	0.003	0.009	0.780	36.935	0.120
MMP10	PE	-0.003	0.010	0.741	42.412	0.030
MMP12	VTE	0.000	0.004	0.965	62.097	0.159
MMP12	DVT	-0.001	0.006	0.826	40.464	0.877
MMP12	PE	0.003	0.007	0.722	82.454	0.005

411

412 MMP, matrix metalloproteinase; VTE, venous thromboembolism; DVT, deep vein thrombosis;

413 PE, pulmonary embolism; SE, standard error

Figure legends

Fig. 1 Three core assumptions of the 2-sample MR design in this study

MMP, matrix metalloproteinase; VTE, venous thromboembolism; DVT, deep vein thrombosis; PE, pulmonary embolism.

Fig. 2 The causal association between MMP and VTE (including DVT and PE) using the inverse-variance weighted mendelian randomization method. A p-value <0.0033 was considered statistically significant. A p-value ranging from 0.0033 to 0.05 was regarded as suggestive evidence.

OR, odds ratio; CI, confidence interval; MMP, matrix metalloproteinase; VTE, venous thromboembolism; DVT, deep vein thrombosis; PE, pulmonary embolism.

Fig. 3 Trends in the causal associations between (A) the MMP-1 level and PE, (B) the MMP-3 level and PE, (C) the MMP-3 level and DVT, (D) the MMP-7 level and PE, (E) the MMP-10 level and VTE, (F) the MMP-12 level and VTE, and (G) the MMP-12 level and PE.

MMP, matrix metalloproteinase; VTE, venous thromboembolism; DVT, deep vein thrombosis; PE, pulmonary embolism.

Table 1. Heterogeneity and pleiotropy test of the significance causal effect of MMPs on VTE

Exposure	Outcome	Pleiotropy			Heterogeneity	
		Intercept	SE	*P*-value	Q	P-value
MMP-1	VTE	-0.005	0.006	0.448	52.521	0.059
MMP-1	DVT	0.007	0.009	0.4 69	54.215	0.053
MMP-1	PE	0.004	0.009	0.658	52.579	0.072
MMP-3	VTE	0.007	0.005	0.148	61.105	0.208
MMP-3	DVT	0.001	0.007	0.841	62.543	0.199
MMP-3	PE	-0.002	0.006	0.760	56.594	0.378
MMP-7	VTE	-0.001	0.007	0.854	21.751	0.194
MMP-7	DVT	0.000	0.009	0.988	11.071	0.853
MMP-7	PE	-0.009	0.010	0.367	20.029	0.219
MMP-10	VTE	0.003	0.007	0.682	38.413	0.072
MMP-10	DVT	0.003	0.009	0.780	36.935	0.120
MMP-10	PE	-0.003	0.010	0.741	42.412	0.030
MMP-12	VTE	0.000	0.004	0.965	62.097	0.159
MMP-12	DVT	-0.001	0.006	0.826	40.464	0.877
MMP-12	PE	0.003	0.007	0.722	82.454	0.005

MMP, matrix metalloproteinase; VTE, venous thromboembolism; DVT, deep vein thrombosis; PE, pulmonary embolism; SE, standard error

Fig. 1 Three core assumptions of the two-

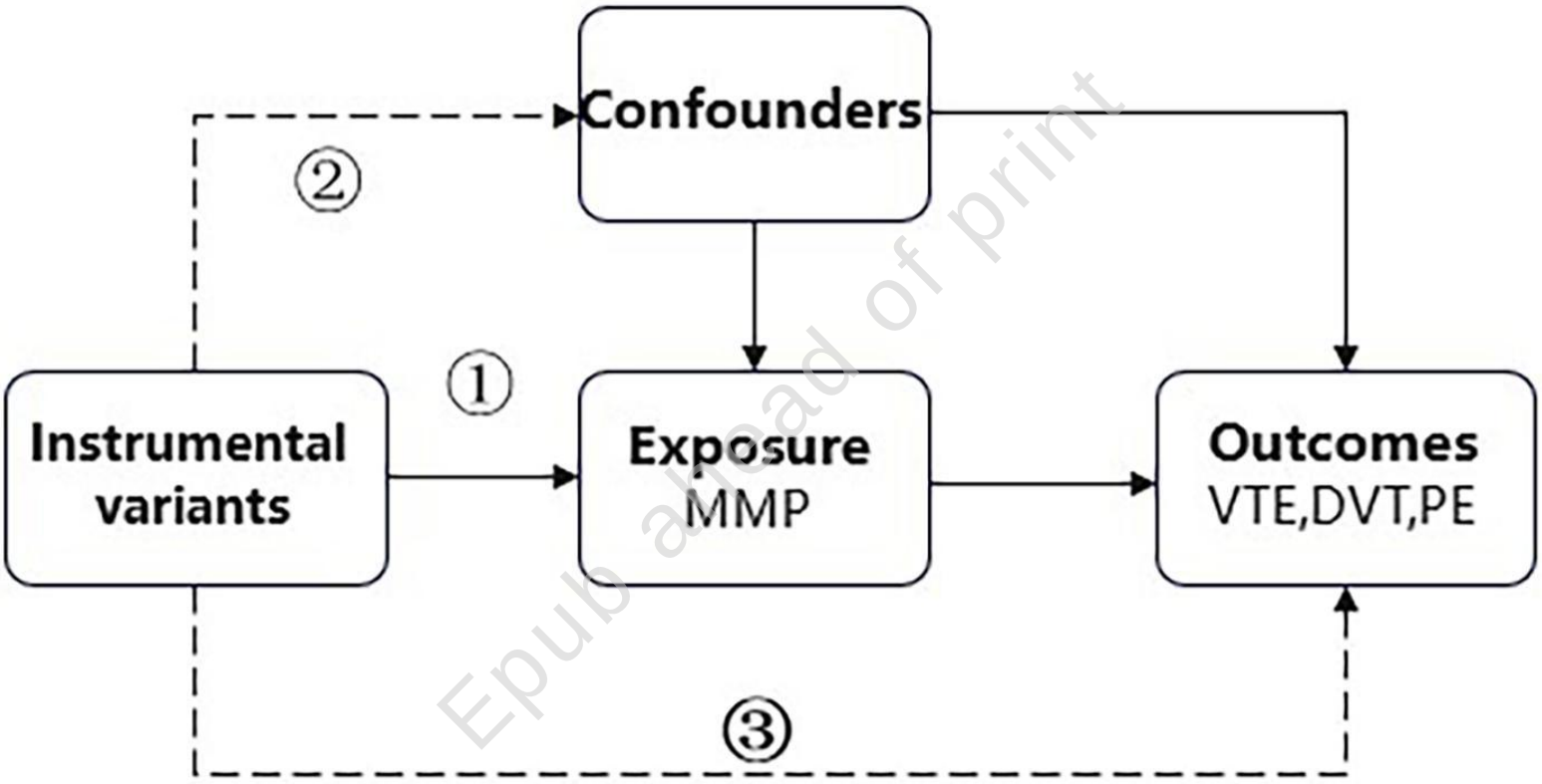

Fig. 2 The causal association between MM

Exposure	Outcome	OR CI (95%)	*P* value
MMP1	VTE	1.02(0.99-1.05)	0.262
MMP1	DVT	1.01(0.97-1.05)	0.724
MMP1	PE	1.06(1.01-1.10)	0.010
MMP3	VTE	1.01(0.98-1.03)	0.594
MMP3	DVT	1.05(1.01-1.09)	0.012
MMP3	PE	0.95(0.92-0.99)	0.006
MMP7	VTE	0.96(0.91-1.01)	0.119
MMP7	DVT	0.98(0.92-1.05)	0.572
MMP7	PE	0.90(0.84-0.97)	0.005
MMP10	VTE	1.04(1.00-1.08)	0.046
MMP10	DVT	1.05(0.99-1.11)	0.099
MMP10	PE	0.98(0.92-1.04)	0.515
MMP12	VTE	**1.04(1.01-1.07)**	**0.002**
MMP12	DVT	1.01(0.98-1.05)	0.399
MMP12	PE	1.06(1.02-1.10)	0.005

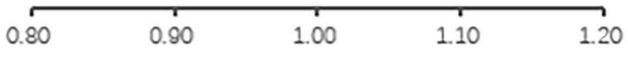

Fig. 3 A. Trend in the causal association be