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# Genetic Evidence for the Causal Link Between Coagulation Factors and the Risk of Ovarian Cancer: A Two-Sample Mendelian Randomization Study

Tiantian Dai 🝺, Yanshuang Jia, Yi Zhang

Department of Obstetrics and Gynecology, Shanghai Changning Maternity and Infant Health Hospital, Shanghai, 200050, People's Republic of China

Correspondence: Yi Zhang, Department of Obstetrics and Gynecology, Shanghai Changning Maternity and Infant Health Hospital, Shanghai, 200050, People's Republic of China, Email m13661903705@163.com

**Background:** Prior investigations have suggested a significant association between coagulation factors and ovarian cancer; however, the precise nature of the causal relationship remains elusive. Our objective is to thoroughly investigate this causal link and delineate the influence of coagulation factors on the risk of ovarian cancer through a rigorous two-sample Mendelian randomization (MR) analysis.

**Methods:** Genetic instrumental variables representing coagulation factors were sourced from four distinct data repositories. Summary statistics pertaining to ovarian cancer were obtained from two extensive Genome-Wide Association Studies (GWAS) for primary and replication analyses, respectively. The primary Mendelian randomization (MR) analysis utilized the inverse-variance weighted (IVW) method. To fortify the reliability of our findings, additional analyses were conducted, including the weighted-median method, MR-Egger regression, MR pleiotropy residual sum and outlier test, Cochran's Q statistic test, MR-Egger intercept analysis, and leave-one-out method, among others.

**Results:** We identified four coagulation factors that were associated with the risk of ovarian cancer in the primary analysis, [odds ratio (OR): 1.365, 95% confidence interval (CI): 1.209–1.542, P <0.001 for von Willebrand factor measurement(vWF); OR: 1.060, 95% CI: 1.018–1.104, P = 0.005 for A disintegrin and metalloproteinase with thrombospondin motifs 13 (ADATMS13); OR: 1.317, 95% CI: 1.002–1.730, P = 0.048 for activated partial thromboplastin time (aPTT); OR: 1.139, 95% CI: 1.063–1.221, P <0.001 for coagulation Factor VIII (FVIII)]. In the meta-analysis, we found that higher levels of coagulation factor VII measurement(FVII) (OR=1.0007, 95% CI: 1.0001–1.0013, P=1.0007) was associated with increased ovarian cancer risk. The results of sensitivity analyses for these coagulation factors were consistent (P<0.05).

**Conclusion:** Our systematic analyses have furnished evidence suggesting a plausible causal association between FVII and the susceptibility to ovarian cancer. Further investigations are warranted to delineate the mechanistic pathways through which coagulation factors influence the progression of ovarian cancer.

Keywords: ovarian cancer, coagulation factor, Mendelian randomization, causal relationship, single nucleotide polymorphism

#### Introduction

Ovarian cancer represents a significant contributor to female cancer-related mortality, largely attributed to delayed diagnosis, with over 70% of cases being identified at advanced stages characterized by extensive abdominal metastasis.<sup>1</sup> Notably, early-stage ovarian cancer diagnosis boasts a five-year survival rate exceeding ~90%, contrasting with a diminished rate of approximately ~45% in advanced-stage diagnoses.<sup>2</sup> Initial symptoms, typically observed in postmenopausal women, include pelvic discomfort and abdominal bloating, indicative of advanced disease stages marked by sizable tumors or ascites.<sup>3</sup> Current therapeutic strategies entail tumor debulking surgery followed by platinum-based chemotherapy,<sup>4</sup> yet recurrence commonly occurs within about 18 months among patients with advanced-stage disease.<sup>1</sup>

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In order to mitigate the elevated mortality rates observed among ovarian cancer patients, there is a pressing need for enhanced understanding of potential pharmacological targets aimed at preventing or managing recurrent and aggressive tumors. Investigations have revealed that a substantial portion of ovarian cancer patients exhibit aberrant levels of hemostatic blood serum markers, suggestive of an activated coagulation system.<sup>5</sup> Additionally, thromboembolic incidents are prevalent among ovarian cancer patients,<sup>6</sup> encompassing both venous thromboembolism and pulmonary embolism.<sup>7</sup> These complications, recognized as Trousseau's syndrome,<sup>8</sup> are prominent among cancer patients, primarily attributed to heightened tissue factor levels.<sup>7</sup> Nonetheless, these observational insights are subject to unmeasured confounders, potentially impeding an accurate portrayal of the interplay between coagulation factors and ovarian cancer. Moreover, reverse causation may introduce bias if modifications or cessation of coagulation factors are influenced by the onset or progression of ovarian cancer.

Mendelian randomization (MR) studies have gained considerable traction in recent years as a methodological approach in etiological research for various diseases. Their prominence in the medical domain has been underscored by their facilitation of causal inference between variables via genetic variation instrumental variables. In the context of lacking randomized controlled trials, Mendelian randomization (MR) offers a compelling approach to investigating causality between exposures and outcomes.<sup>9</sup> MR effectively emulates randomized controlled trials through the utilization of single-nucleotide polymorphisms (SNPs) associated with exposures as instrumental variables (IVs).<sup>10</sup> The instrumental variable (IV) methodology mirrors randomization by virtue of single-nucleotide polymorphisms (SNPs) being randomly assigned to offspring upon conception, thus attenuating the impact of confounding variables. Attributes at the individual level, such as gender and age, are rendered less liable to distort causal effects.<sup>11,12</sup> Moreover, the formation of genotypes precedes disease onset, reducing the likelihood of errors related to reverse causality in MR studies.<sup>12</sup>

In this study, we specifically chose 11 coagulation factors—coagulation Factor XI (FXI), coagulation factor VII (FVII), coagulation Factor X (FX), coagulation Factor VIII (FVIII), von Willebrand factor (vWF), plasminogen activator inhibitor 1 (PAI-1), activated partial thromboplastin time (aPTT), plasmin, activated protein C, a disintegrin and metalloproteinase with thrombospondin motifs 13 (ADAMTS13), and prothrombin—as exposures, while utilizing ovarian cancer as the outcome for Mendelian randomization (MR) analysis. Our objective was to investigate the potential causal link between coagulation factors and ovarian cancer, aiming to establish a conceptual framework for further exploration into the intricate mechanisms and risk factors associated with ovarian cancer.

# **Materials and Methods**

#### Study Design

Figure 1 illustrates the schematic representation of the study design. We utilized a two-sample Mendelian randomization (MR) framework to investigate the causal connections between 11 coagulation factors and ovarian cancer. To obtain the necessary data, we collected summary statistics from genome-wide association studies (GWAS) available in public repositories, thereby bypassing the requirement for institutional review board approval or informed consent, as elaborated in <u>Supplementary Table 1</u>. A robust MR analysis adheres to three core assumptions: (1) a strong and consistent correlation between instrumental variables and the exposure factor; (2) independence between instrumental variables and confounding factors affecting the relationship between exposure and outcome; and (3) genetic variations influencing the outcome solely through the exposure factor and not through alternate pathways.<sup>14</sup>

In this investigation, we employed Mendelian randomization (MR) analysis utilizing data derived from two distinct ovarian cancer datasets. The conventional inverse variance weighted (IVW)<sup>15</sup> method was applied to assess the causal impact of 11 coagulation factors on ovarian cancer. Furthermore, we conducted four supplementary analyses, including weighted median,<sup>16</sup> weighted mode,<sup>17</sup> simple mode<sup>18</sup> and MR-Egger.<sup>19</sup> To enhance the robustness of our findings, a meta-analysis was conducted by amalgamating results from the two ovarian cancer datasets. Phelan et al<sup>13</sup> dataset served as the primary discovery dataset, and to validate our findings, we utilized GWAS summary statistics from UK Biobank<sup>20</sup> for replication. Adhering to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline for MR, our study maintained rigorous standards in its methodology and reporting.<sup>21</sup>



Figure I Sketch map for the overall design of the Mendelian randomization study.

## **GWAS** Summary Statistics

All statistics involved in the analysis were derived from publicly available large-scale GWASs, as shown in <u>Supplementary Table 1</u>. In our study, we included 11 coagulation factors from four distinct data source as described below. Also, to ensure the robustness of the outcomes, we implemented two different ovarian cancer datasets.

## Exposure Data of 11 Coagulation Factors

The comprehensive GWAS summary statistics data on Coagulation Factor XI (FXI), Coagulation factor VII (FVII), Coagulation Factor X (FX), Coagulation Factor VIII (FVIII), von Willebrand factor measurement (vWF) and Plasminogen activator inhibitor 1 (PAI-1) originate from genome-wide association scans involving 4775 proteins as documented in the Fenland database. These scans were conducted on a cohort comprising 10,708 participants, whose blood protein profiles were obtained through the SomaScan version 4 assay.<sup>22</sup> Summary statistics for A disintegrin and metalloproteinase with thrombospondin motifs 13 levels (ADATMS13) was derived from a meta-analysis of whole-genome sequencing (WGS) data sourced from two Greek cohorts, MANOLIS (n = 1356) and Pomak (n = 1537). These cohorts undertook a protein quantitative trait locus (pQTL) analysis encompassing 248 serum proteins across a total of 2893 individuals.<sup>23</sup> Summary statistics for aPTT were acquired from the BioVU biobank, encompassing 94,474 individuals of diverse ancestral and racial backgrounds genotyped using the Illumina MEGAEX array.<sup>24</sup> Furthermore, summary statistics pertaining to plasmin, activated protein C, and Prothrombin were sourced from the INTERVAL study, designed primarily to ascertain the optimal interval between blood donations. The proteomic profiles were derived from 3301 blood donors, encompassing 3622 plasma proteins quantified via SOMAscan (SomaLogic, Inc).<sup>25</sup> These

coagulation factors could be categorized into five groups, including platelet adhesion (vWF and ADAMTS13), intrinsic pathway (FXI, aPTT, and FVIII), extrinsic pathway (FVII), common pathways (prothrombin and FX), and dissolution of fibrin clot (PAI-1, protein C, and plasmin).

# Outcome Data of Ovarian Cancer

The summary data for ovarian cancer were retrieved from two data source. In the preliminary phase, we utilized the genome-wide association statistics conducted by Phelan et al<sup>13</sup> which meta-analyzed the OncoArray, COGS and other five GWAS datasets, with 25,509 controls and 40,941 ovarian cancer cases. To validate our findings, we utilized the genome-wide association derived from UK Biobank,<sup>20</sup> analyses were restricted to individuals of self-reported European ancestry, ensuring concordance between self-reported and genetic sex. Within this GWAS, total 199,741 European participants were included, with 198,523 controls and 1218 ovarian cancer cases.

## Genetic Instruments Selection and Harmonization

To ascertain the credibility and precision of the findings, a rigorous assessment of single-nucleotide polymorphisms (SNPs) was undertaken to derive reliable instrumental variables (IVs). The criteria for SNP selection were delineated as follows: Firstly, SNPs were required to demonstrate a robust correlation with the exposure. Secondly, SNPs were mandated to be independent of any confounding factors. Lastly, SNPs were expected to be associated with outcomes influenced by the exposure.<sup>26</sup> A set of selection criteria was systematically applied to identify suitable genetic instrumental variables (IVs) for 11 coagulation factors, namely FXI, FVII, FX, FVIII, vWF, ADATMS13, aPTT, plasmin, activated protein C, prothrombin, and PAI-1: (1) Single-nucleotide polymorphisms (SNPs) demonstrating robust associations with the coagulation factors were chosen as candidate instrumental variables (IVs) at a significance level of p < 5e-8. Due to the restricted availability of IVs meeting the genome-wide significance threshold (p < 5e-8), notably for aPTT and prothrombin, a more lenient threshold (p < 5e-6) was applied. This adjustment aimed to encompass potential variant sets with enriched associations, thus facilitating a broader exploration and comprehensive analysis. (2) To maintain the autonomy of each single-nucleotide polymorphism (SNP), a rigorous linkage disequilibrium (LD) clumping criterion (r2 < 0.001 and window = 10000 kb) was utilized in the selection process of genetic instruments. This approach was implemented through the "clump data" function integrated into the TwoSampleMR R package, ensuring the exclusion of SNPs in close LD proximity to each other. (3) Harmonization of data was performed to integrate SNP-coagulation factor associations and SNP-ovarian cancer associations, employing the "harmonise data" function in the TwoSampleMR R package. SNPs with a minor allele frequency of < 0.01, ambiguous SNPs displaying non-concordant alleles, and those demonstrating palindromic patterns with intermediate allele frequencies were systematically excluded from the analysis. Allele information was utilized to infer the forward strand alleles accurately. (4) A thorough investigation was carried out using PhenoScanner<sup>27,28</sup> to identify all documented phenotypes linked with the genetic instrumental variables (IVs). IVs correlated with any other established phenotype were consequently omitted from subsequent Mendelian randomization (MR) analysis. (5) Following this, F statistics were computed to assess the robustness of the selected single-nucleotide polymorphisms (SNPs) using the subsequent formula:  $R^2=2 \times MAF \times (1 - MAF) \times \beta^2$ ,  $F=R^2 (n-k-1) / k(1-R^2)$ .<sup>29,30</sup> In this equation, "MAF" represents the minor allele frequency of SNPs employed as IVs, the variable R2 denotes the fraction of variance explained by each single-nucleotide polymorphism (SNP), where N represents the sample size of the genome-wide association study (GWAS), and K denotes the number of SNPs under consideration. An F statistic exceeding 10 suggests the absence of compelling evidence of instrument bias.<sup>31</sup>

## Statistical Analysis and Sensitivity Analysis

In both the initial and replication phases, we investigated the causal association between the 11 coagulation factors and ovarian cancer employing various methodologies, including inverse variance weighted (IVW) or Wald ratio, MR-Egger, weighted median, weighted mode, and simple mode.

The Wald ratio estimate for each single-nucleotide polymorphism (SNP) was calculated by dividing the per-allele effect on ovarian cancer by the per-allele change in the log odds of coagulation factors. Subsequently, an aggregated causal effect was obtained by meta-analyzing the Wald ratio estimates using the inverse variance-weighted (IVW)

method. The IVW method is recognized as the most accurate and robust technique for estimating causal effects, particularly when all chosen SNPs act as valid instrumental variables. When only one single-nucleotide polymorphism (SNP) was available, Wald ratios were applied, while analyses incorporating three or more genetic instruments utilized the fixed-effects inverse variance-weighted (IVW) method or the random-effects IVW<sup>15</sup> method as the principal Mendelian randomization (MR) analytical approach to assess the causal influence of genetically predicted circulating levels of coagulation factors on ovarian cancer risk.

The primary analytical method employed was the inverse-variance weighted (IVW) method. Moreover, for certain coagulation factors furnished with four or more genetic instruments, a spectrum of supplementary analyses was employed to scrutinize the robustness of the IVW findings against potential violations. The analyses encompassed MR-Egger, weighted median, weighted mode, and simple mode methodologies. Notably, the MR-Egger method resembles the random-effects IVW mode but does not impose a constraint on the intercept.<sup>19,32</sup> A non-zero MR-Egger's intercept signals the potential existence of horizontal pleiotropy. Conversely, the weighted median method furnishes reliable Mendelian randomization (MR) estimates under the assumption that more than 50% of weights derive from valid instruments.<sup>16</sup> The mode-based methods (simple mode and weighted mode) operate under the assumption that the most prevalent causal effect aligns with the true causal effect, enabling certain instruments to be invalid without introducing bias to the estimated causal effect.<sup>17,18</sup>

For sensitivity analysis, the Cochran's Q statistic was utilized to assess heterogeneity, with a Cochran Q-derived test p-value < 0.05 considered indicative of substantial heterogeneity. In the presence of observed heterogeneity, the randomeffects IVW method was employed in lieu of the fixed-effect IVW.<sup>33</sup> MR-Egger was utilized to address uncorrelated pleiotropy arising from SNPs acting as instrumental variables. To address the potential influence of horizontal pleiotropy, MR-Egger regression was employed to evaluate pleiotropy through the intercept term.<sup>19</sup> A p-value below 0.05 for the intercept suggested the existence of horizontal pleiotropy. Additionally, the MR-PRESSO method was employed to detect and exclude potential outliers influenced by horizontal pleiotropy. Following the removal of outliers, the data underwent re-analysis.<sup>34</sup> Furthermore, scatter plots, forest plots, and funnel plots were employed to identify outliers and discern evident heterogeneity. Additionally, a leave-one-out analysis was conducted to assess the potential influence or bias of individual SNPs on the Mendelian randomization estimate.<sup>35</sup>

#### **Replication and Meta-Analysis**

To enhance the statistical robustness and precision of causal estimates, we incorporated genome-wide association study (GWAS) summary statistics from the UK Biobank<sup>20</sup> for replication purposes. A fixed-effect or random-effect metaanalysis was conducted to amalgamate the causal estimates obtained from both the initial and replication phases, utilizing the metafor R package.<sup>36</sup>

The relationship between coagulation factors and ovarian cancer was depicted through odds ratios (ORs) accompanied by 95% confidence intervals (CIs). A significance threshold of P < 0.05 was applied for all analyses. The aforementioned analyses were carried out utilizing the "TwoSampleMR (version 0.5.6)", "MRPRESSO (version 1.0)", and "metafor (version 4.4–0)" packages within R version 4.3.2.

#### Results

#### Preliminary Analysis

Following stringent quality control measures for instrumental variables (IVs), we comprehensively investigated 11 coagulation factors in our Mendelian randomization (MR) study. In our analysis, the number of IVs varied from 1 to 17 for each coagulation factor. Detailed information regarding the instrumental variables of coagulation factors and ovarian cancer is provided in <u>Supplementary Table 2</u>. The F-statistics for all SNPs included in our analysis ranged from 21.1 to 6085.9, all surpassing the threshold of 10, indicating a reduced likelihood of weak instrument bias (Supplementary Table 2).

We detected statistically significant evidence suggesting a potential causal relationship between four coagulation factors and the risk of ovarian cancer utilizing the IVW method. These factors include von Willebrand factor (vWF),

ADAMTS13, activated partial thromboplastin time (aPTT), and coagulation Factor VIII (FVIII). The specifics of the single-nucleotide polymorphisms (SNPs) utilized for the coagulation factors are provided in <u>Supplementary Table 2</u>, while the outcomes of the associations between these four coagulation factors and the risk of ovarian cancer are delineated in Figure 2 and <u>Supplementary Table 3</u>. As depicted in Figure 2, these four coagulation factors are categorized into platelet adhesion and intrinsic pathways.

We identified positive associations between von Willebrand factor (vWF) (OR: 1.365, 95% CI: 1.209–1.542, P <0.001), ADAMTS13 (OR: 1.060, 95% CI: 1.018–1.104, P = 0.005), activated partial thromboplastin time (aPTT) (OR: 1.317, 95% CI: 1.002–1.730, P = 0.048), and coagulation Factor VIII (FVIII) (OR: 1.139, 95% CI: 1.063–1.221, P <0.001) with the risk of ovarian cancer, as determined by the IVW or Wald ratio method (Supplementary Table 3). Consistent findings were observed with complementary Mendelian randomization (MR) methods. The MR-PRESSO test did not identify any outliers, and the association estimate remained consistent. MR-Egger regression indicated minimal evidence of directional pleiotropy (P\_intercept > 0.05). Cochran's Q statistic provided strong evidence of homogeneity (P > 0.05) except for FVIII, for which we employed the random-effects IVW method. Leave-one-out analysis, scatter plots, funnel plots, and forest plots collectively supported the robustness of the MR estimates (Figures S1–S4).

#### **Replication and Meta-Analysis**

To enhance the robustness of our findings, we conducted a replication analysis using the GWAS dataset from UK Biobank for ovarian cancer, as depicted in Figure 3. Our analysis revealed a positive association between coagulation Factor VII (FVII) and the risk of ovarian cancer (OR: 1.00070, 95% CI: 1.00011–1.00128, P = 0.02) using the IVW method (Supplementary Table 3). Although a consistent trend was observed for other candidate coagulation factors in the alternate GWAS dataset, the results did not attain statistical significance, likely attributed to substantial differences in sample size. Specifically, von Willebrand factor (vWF) (OR: 1.00095, 95% CI: 0.99786–1.00405, P = 0.549), ADAMTS13 (OR: 1.00039, 95% CI: 0.99967–1.00111, P = 0.286), activated partial thromboplastin time (aPTT) (OR: 1.00103, 95% CI: 0.99564–1.00644, P = 0.709), and coagulation Factor VIII (FVIII) (OR: 0.99950, 95% CI: 0.99700–1.00199, P = 0.692) exhibited similar trends based on the IVW method (Supplementary Table 3). Complementary MR analyses yielded consistent results. The MR-PRESSO test did not identify any outliers, and the association estimates remained consistent. Minimal evidence of directional pleiotropy was detected by MR-Egger regression (P\_intercept > 0.05). Cochran's Q statistic provided strong evidence of homogeneity (P > 0.05) except for

Exposure	Method	nSNP	P-Value			OR (95% CI)
Platelet adhesion						
vWF	Wald ratio	1	<0.001		_ <b>_</b>	• 1.365 (1.209 to 1.542)
ADATMS13	IVW	6	0.005		-	1.060 (1.018 to 1.104)
Intrinsic pathways						
aPTT	IVW	9	0.048		<b></b> >	• 1.317 (1.002 to 1.730)
FVIII	IVW	7	<0.001			1.139 (1.063 to 1.221)
FXI	IVW	5	0.211		-	1.026 (0.985 to 1.069)
Extrinsic pathways						
FVII	IVW	5	0.979		- <b>-</b>	0.999 (0.955 to 1.045)
Common pathways						
FX	IVW	10	0.426			0.975 (0.917 to 1.037)
Prothrombin	IVW	17	0.814			1.008 (0.944 to 1.075)
Dissolution of fibrin clot						
PAI-1	IVW	3	0.583	_		1.065 (0.851 to 1.332)
Plasmin	IVW	3	0.736			1.018 (0.919 to 1.127)
Activated Protein C	IVW	2	0.773	-		1.018 (0.901 to 1.151)
				0.5	1 1	5

Figure 2 Mendelian randomization analyses of coagulation factors on ovarian cancer using outcome from Phelan et al<sup>13</sup> dataset.

Exposure	Method	nSNP	P-Valu	e		OR (95% CI)
Platelet adhesion						
vWF	IVW	3	0.549			1.00095 (0.99786 to 1.00405)
ADATMS13	IVW	5	0.286	_	-	1.00039 (0.99967 to 1.00111)
Intrinsic pathways						
aPTT	IVW	7	0.709			1.00103 (0.99564 to 1.00644)
FVIII	IVW	8	0.692			0.99950 (0.99700 to 1.00199)
FXI	IVW	3	0.802	-	-	1.00010 (0.99935 to 1.00084)
Extrinsic pathways						
FVII	IVW	4	0.02		<b></b>	1.00070 (1.00011 to 1.00128)
Common pathways						
FX	IVW	10	0.95	-	-	1.00003 (0.99895 to 1.00112)
Prothrombin	IVW	16	0.625	-	-	1.00030 (0.99910 to 1.00149)
Dissolution of fibrin clot						
PAI-1	IVW	3	0.873	<b>_</b>		0.99976 (0.99677 to 1.00275)
Plasmin	IVW	2	0.383	_	-	1.00094 (0.99883 to 1.00306)
Activated Protein C	IVW	2	0.694			0.99941 (0.99646 to 1.00237)
				0.99	1 1.0	)1

Figure 3 Mendelian randomization analyses of coagulation factors on ovarian cancer using outcome from UK Biobank.

Exposure	Study	P-Valu	е		OR (95% CI)
aPTT	Phelan	0.048			→ 1.3169 (1.0025 to 1.7300)
	Burrows	0.709			1.0010 (0.9956 to 1.0064)
	Meta	0.438			1.1083 (0.8548 to 1.4371)
FVIII	Phelan	<0.001		_ <b></b>	1.1390 (1.0627 to 1.2208)
	Burrows	0.692			0.9995 (0.9970 to 1.0020)
	Meta	0.357			1.0619 (0.9346 to 1.2065)
FVII	Phelan	0.979			0.9994 (0.9555 to 1.0453)
	Burrows	0.02		+	1.0007 (1.0001 to 1.0013)
	Meta	0.021		÷	1.0007 (1.0001 to 1.0013)
ADATMS13	Phelan	0.005		-=-	1.0602 (1.0180 to 1.1041)
	Burrows	0.286		÷	1.0004 (0.9997 to 1.0011)
	Meta	0.372			1.0261 (0.9698 to 1.0856)
			0.5	1	1.5

Figure 4 Summary statistics of the causal estimates of coagulation factors on ovarian cancer in the meta-analysis.

FVIII, for which we utilized the result of the random-effects IVW method. Leave-one-out analysis, scatter plots, funnel plots, and forest plots collectively supported the robustness of the MR estimates (Figures S5–S8).

Due to the heterogeneity of the methods used for MR analysis, it's impossible to conduct meta-analysis for the MR results of vWF. The meta-analysis identified 1 coagulation factor with potential effects on ovarian cancer, as shown in Figure 4. Higher levels of FVII (OR=1.0007, 95% CI: 1.0001–1.0013, P=1.0007) was associated with increased ovarian cancer risk. Therefore, through meta-analysis, we identified FVII causally linked to ovarian cancer.

## Discussion

To our understanding, this study marks the first exploration of Mendelian randomization (MR) that combines coagulation factors and ovarian cancer. In this investigation, we merged data from two comprehensive genome-wide association studies (GWAS) to thoroughly assess the causal implications of genetically proxied 11 coagulation factors on the development of ovarian cancer. Through this comprehensive two-sample MR analysis, we discerned a notable association between coagulation factor VII (FVII) and the susceptibility to ovarian cancer, thereby paving the way for deeper

exploration into the intricate roles of coagulation factors in ovarian carcinogenesis. Furthermore, these findings furnish valuable insights for guiding future intervention strategies and the identification of potential therapeutic targets.

Coagulation, recognized as a hallmark of tumor progression, can stem from augmented plasma extravasation and vascular permeability, leading to extravascular coagulation, or from vessel disruption, resulting in intravascular coagulation.<sup>37</sup> Patients afflicted with malignant tumors are predisposed to coagulation abnormalities, including cancerassociated thrombosis.<sup>38</sup> There is emerging evidence suggesting that tumor cells possess the capability to release procoagulant factors, such as tissue factors, potentially instigating coagulation cascades. Conversely, tumor coagulum, a network of molecular effectors influenced by cancer, can interact with the tumor microenvironment (TME), impacting cancer progression or inhibition.<sup>39</sup> Fundamentally, activation of the clotting system aids in cancer advancement by participating in various processes including tumor growth, angiogenesis, invasion, immune evasion, and metastasis, Coagulation factors contribute to these malignant processes both dependently (through fibrin clot formation, platelet recruitment, immune response regulation, and tissue factor (TF) + microparticles (MPs) secretion) and independently (via intracellular signaling).<sup>5</sup> Recent investigations have linked poor prognosis in ovarian cancer patients with disorders in the coagulation system.<sup>40</sup> While prior research has elucidated the involvement of coagulation factors in the biological mechanisms of ovarian cancer, their potential for early screening and prevention remains constrained by the unclear causal relationship between the two. Hence, we undertook a critical Mendelian randomization (MR) study to elucidate the causal connection between coagulation factors and ovarian cancer, with the aim of providing insights for ovarian cancer screening and treatment strategies.

In this study, Factor VII (FVII) has been identified as a contributory factor to the susceptibility of ovarian cancer. FVII, a precursor serine protease biosynthesized in the liver and subsequently released into the circulatory system, assumes a pivotal role in orchestrating the blood coagulation cascade. Tissue factor (TF), a transmembrane glycoprotein expressed in both physiological and neoplastic tissues, functions as the receptor for FVII. Upon engagement, TF instigates the extrinsic blood coagulation pathway, thereby instigating the generation of activated factor X (FXa) through the conversion of FVII to its activated form, FVIIa.<sup>41</sup> The serine protease activity intrinsic to FVIIa within the TF-FVIIa complex precipitates a cascade of enzymatic events, culminating in the formation of blood clots comprised of platelets and erythrocytes enveloped in fibrin polymers.<sup>42-44</sup> Significantly, the TF-FVII complex manifests abnormal expression patterns on the surface of neoplastic cells, including those originating from ovarian tissues. This procoagulant complex elicits the initiation of intracellular signaling cascades, thereby contributing to the acquisition of malignant characteristics. Given the frequent exposure of cancerous tissues to hypoxic microenvironments, the expression of TF and FVII can be induced at the genetic level in ovarian cancer cells in response to hypoxia, thereby prompting the autonomous generation of the TF-FVII complex.<sup>7</sup> Additionally, a physical interaction between the ubiquitously expressed transcription factor Sp1 and HIF2 emerges as a primary mechanism driving FVII gene activation under hypoxic conditions, particularly in ovarian clear cell carcinoma (CCC) cells with poor prognostic outcomes. Furthermore, the activation of FVII is synergistically potentiated under hypoxic conditions when cells are cultured under serum starvation.<sup>45</sup>

Our Mendelian randomization (MR) inquiry demonstrates several significant strengths. Firstly, it elucidates the causal nexus between coagulation factors and ovarian cancer, thereby enriching our comprehension of the nuanced mechanisms governing their interplay. This methodological framework permits an exhaustive exploration of the diverse impacts of coagulation factors on cancer genesis. Secondly, to mitigate potential biases arising from weak instrumental variables, we executed two-sample MR analyses employing distinct datasets for the exposure (coagulation factors) and outcome (ovarian cancer) variables. Thirdly, we capitalized on data sourced from two independent large-scale cohorts for MR investigations, subsequently subjecting them to meta-analysis, thereby ensuring a robust sample size for the outcome variable and amplifying the generalizability of our findings. Fourthly, we conducted supplementary analyses encompassing evaluations of heterogeneity, pleiotropy, and leave-one-out sensitivity to corroborate the assumptions underlying our utilization of instrumental variables. These endeavors fortify the credibility and resilience of the conclusions drawn from our study.

Nevertheless, our Mendelian randomization (MR) study is not without its limitations. Firstly, while we have established causal relationships between exposures and outcomes, the precise magnitude of these associations may be subject to uncertainty. Thus, further investigations are warranted to validate our results rigorously. Secondly, our study is

predominantly based on data analysis, lacking direct experimental validation in animal models or validation in patient cohorts. Subsequent experimental validation is essential to confirm our conclusions robustly. Thirdly, within the specific domain of ovarian cancer as a condition predominantly affecting females, it is noteworthy that prior Genome-Wide Association Studies (GWASs) exploring diverse coagulation factors have exhibited a tendency towards sampling from combined-sex populations. Given the requisite consistency in population characteristics for both sample sets in two-sample MR analyses, potential incongruities in genetic estimations of coagulation factors across genders necessitate attention, as such discrepancies could introduce bias into our MR findings. Fourthly, owing to the confinement of our study to individuals of European descent, the applicability of our findings to other demographic groups may be restricted. Therefore, further investigation into the causal relationships between coagulation factors and ovarian cancer across diverse populations is imperative.

In conclusion, our investigation confirms the causal influence of FVII on ovarian cancer risk, reinstating its significance in the pathogenesis of the disease. These results hold promise for shedding light on the underlying mechanisms and identifying novel therapeutic avenues for ovarian cancer. Our study significantly enhances comprehension of the role of coagulation cascades in ovarian cancer development, potentially guiding future strategies for prevention and treatment.

## **Data Sharing Statement**

The genotype and phenotype data are available on application from the GWAS Catalog or the published article and its Supplementary Files, the IEU Open GWAS (https://gwas.mrcieu.ac.uk/), UK Biobank (https://gwas.mrcieu.ac.uk/).

## **Ethics Approval and Consent to Participate**

The study protocol was approved by the ethics committee of Shanghai Changning Maternity and Infant Health Hospital (CNFBLLAR-2024–006).

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# **Author Contributions**

Tiantian Dai was the first author. Yi Zhang was the corresponding author. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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# Disclosure

The authors declare no conflicts of interest in this work.

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