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ORIGINAL ARTICLE

High plasma levels of C1-inhibitor are associated with lower risk of future venous thromboembolism

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Abstract

Background: C1-inhibitor (C1INH) is a broad-acting serine protease inhibitor with anticoagulant activity. The impact of C1INH plasma levels within the normal physiological range on risk of venous thromboembolism (VTE) is unknown. We assessed the association of plasma C1INH levels and VTE risk and evaluated the impact of C1INH on thrombin and plasmin generation in *ex vivo* assays.

Methods: A nested case-control study with 405 patients with VTE and 829 age- and sex-matched controls was derived from the Tromsø Study. Odds ratios (ORs) with 95% confidence intervals (95% CI) for VTE were estimated across plasma C1INH quartiles. Genetic regulation of C1INH was explored using quantitative trait loci analysis of whole exome sequencing data. The effect of plasma C1INH levels on coagulation was evaluated *ex vivo* by calibrated automated thrombography.

Results: Individuals with C1INH levels in the highest quartile had a lower risk of VTE (OR 0.68, 95% CI: 0.49-0.96) compared with those with C1INH in the lowest quartile. In subgroup analysis, the corresponding ORs were 0.60 (95% CI: 0.39-0.89) for deep vein thrombosis and 0.85 (95% CI: 0.52-1.38) for pulmonary embolism, respectively. No significant genetic determinants of plasma C1INH levels were identified. Addition of exogenous C1INH to normal human plasma reduced thrombin generation triggered by an activator of the intrinsic coagulation pathway, but not when triggered by an activator of the extrinsic coagulation pathway.

Conclusions: High plasma levels of C1INH were associated with lower risk of VTE, and C1INH inhibited thrombin generation initiated by the intrinsic coagulation pathway *ex vivo*.

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KEYWORDS
C1 inhibitor, complement system, risk, thrombin generation, venous thromboembolism

1 | INTRODUCTION

Venous thromboembolism (VTE), encompassing deep vein thrombosis (DVT), and pulmonary embolism (PE) is a common cardiovascular disease [1,2] with severe complications, such as post-thrombotic syndrome, post-PE syndrome, recurrence, disability, and death [3–6]. The etiology of VTE is complex, and several provoking factors contribute to VTE risk, including immobilization, malignancy, surgery, and hospitalization [7,8]. However, up to 50% of the VTE events occur without an apparent provoking factor and are considered unprovoked [9]. In contrast to time trends in arterial thrombosis, which have shown a substantial decline during the past decades [10,11], the incidence of VTE in the general population has not declined in the same period [12–14]. Thus, there is a need to discover novel biomarkers of VTE to improve risk assessment and provide further insights into causative disease mechanisms of VTE.

An increasing body of evidence has implicated a role for the intrinsic pathway of coagulation in the pathogenesis of VTE [15]. Individuals with elevated plasma levels of factor (F) XI are at increased risk of VTE [16], whereas individuals with severe deficiency of FXI have decreased risk of VTE [17]. Moreover, FXI inhibitors have been shown to effectively prevent VTE in patients exposed to orthopedic surgery [18–21]. C1-inhibitor (C1INH) is a serine protease inhibitor that inhibits the activated form of several components of the complement pathway, including the C1qrs complex and the mannose-binding lectin-associated serine proteases 1 and 2 (MASP-1 and 2) [22]. Importantly, C1INH also serves as a major physiological inhibitor of FXIIa, plasma kallikrein, and FXIa [23,24], as well as serine proteases involved in the fibrinolytic systems [25,26].

It was recently reported that C1INH deficiency, resulting in hereditary angioedema, is associated with an increased risk of VTE [27,28]. The impact of C1INH plasma levels within the normal physiological range on VTE risk is unknown. High C1INH levels may protect against VTE by inhibition of the intrinsic coagulation system [23,24]. Alternatively, high C1INH levels may increase the risk of VTE by inhibition of fibrinolysis [29–31]. Recently, we conducted a small untargeted mass spectrometry (MS)-based plasma proteomic study to identify novel biomarkers of VTE and found an inverse association between plasma C1INH levels and VTE risk [32]. In this study, we (i) investigated the association of plasma C1INH levels and VTE risk in a large, nested case-control study, (ii) investigated to what extent plasma C1INH levels were genetically regulated using protein quantitative trait loci (pQTL) analyses, and (iii) evaluated the impact of exogenous added C1INH on thrombin and plasmin generation in *ex vivo* assays.

Essentials

- C1-inhibitor (C1INH) is a broad-acting serine protease inhibitor with anticoagulant functions.
- We investigated the association of plasma C1INH levels with risk of venous thromboembolism (VTE).
- Increasing plasma levels of C1INH were associated with reduced risk of VTE.
- C1INH inhibited thrombin generation initiated by the intrinsic coagulation pathway *ex vivo*.

2 | METHODS

2.1 | The Tromsø Study

Participants were recruited from the fourth survey of the Tromsø Study (Tromsø 4), a population-based cohort of 27 158 participants, aged 25 to 97 years, with inclusion in 1994/1995 and follow-up until September 2007 [33]. All participants provided written informed consent, and the regional committee for medical research ethics approved the study. As previously described [2,34], all first-lifetime, objectively confirmed VTE events occurring among the participants during follow-up were identified and adjudicated by extensive review of medical records at the University Hospital of North Norway, which is the sole hospital covering in- and outpatient care of VTE in the entire Tromsø region. In total, 462 VTE events occurred during the follow-up period. The VTEs were classified as either PE (including those with and without concurrent DVT) or DVT, and as provoked or unprovoked (for definitions of provoking factors, please see Supplementary Material).

2.1.1 | Baseline measurements and plasma collection in the Tromsø Study

Baseline characteristics were collected by physical examination and self-administered questionnaires at study enrollment in 1994/1995 [33]. Height and weight were measured and body mass index (BMI) was calculated as kg/m^2. Information on comorbidities (cancer, diabetes, cardiovascular disease) at inclusion in the cohort was obtained from the questionnaires. Nonfasting blood samples were drawn into 5-mL vacutainer tubes containing ethylenediaminetetraacetic acid as an anticoagulant (K3- ethylenediaminetetraacetic acid 40 µL, 0.37 mol/L per tube). Samples were processed within 1 hour by centrifugation at 3000 × *g* for 10 minutes at 22 °C, and plasma was stored 1 mL aliquots at −70 °C by the Tromsø Study.

2.2 | Step 1 – untargeted proteomics case-control study

From the Tromsø 4 cohort, we had previously established a nested case-control study of 100 VTE cases and 100 controls [32], with blood samples collected at cohort inclusion (ie, before VTE). Cases were prioritized according to the shortest follow-up time from blood sampling to VTE, and for each VTE case, 1 age- and sex-matched control was randomly sampled from the parent cohort [32]. MS-based untargeted plasma proteome analysis was performed at Proteomic Sciences (Cobham, United Kingdom). A detailed description of sample preparation, generation of MS raw data, data processing, and statistical analyses performed to identify biomarker candidates for VTE is provided elsewhere [32]. As previously described, 24 of the samples were excluded because of preanalytical conditions [32], thus rendering 87 VTE cases and 89 controls eligible for analysis. In the proteome-wide analysis, we found an inverse association between plasma C1INH levels and VTE risk (these results can be found in the Supplementary Material of Jensen et al. [32]), but the association was not further explored. For the present study, we investigated this association in more detail and categorized the study population based on the quartile cut-offs of C1INH plasma levels determined in the controls (ie, semiquantitative levels of C1INH obtained from MS), and estimated odds ratios (ORs) of VTE with 95% CI adjusted for age, sex, BMI, and cancer across quartiles of C1INH.

2.3 | Step 2 – association between C1INH and VTE in a large, nested case-control study

To extend our findings from untargeted proteomics in a larger population and with quantitative measures of C1INH, we created a nested case-control study of all VTE cases (n = 462) and 2 age- and sex-matched controls (n = 924) from the Tromsø 4 cohort (follow-up: 1994-2007). In a nested case-control study, the temporal sequence between exposure (eg, C1INH) and outcome (eg, VTE) is preserved because blood samples were collected at cohort inclusion, and this design is therefore efficient to study biological precursors of disease. A total of 57 cases and 95 controls were excluded because plasma samples were not available, leaving 405 cases and 829 controls for analysis of C1INH. A flowchart describing the study population from untargeted proteomics (Step 1) and nested case-control (Step 2) studies is shown in Figure 1.

Stored plasma samples were thawed in a water bath at 37 °C for 5 minutes, followed by centrifugation for 2 minutes at 13 500 × *g* to obtain platelet-free plasma. C1INH antigen levels were determined in duplicate by enzyme-immunoassays (EIA) using commercially available antibodies (DY2488; R&D Systems) with intra-assay coefficients of variation of 4.5% and interassay CV of 9.8%. C-reactive protein (CRP) was used as a proxy for general inflammation, and was measured by the high-sensitive technique by EIA using commercially available reagents (R&D Systems) as previously described [35]. For more details on the EIA methods, please see Supplementary Material.

Statistical analyses were carried out using Stata version 16 (StataCorp LLC) and R version 4 (The R Foundation for Statistical Computing, Vienna, Austria. https://cran.r-project.org). Plasma C1INH was categorized according to quartile cut-offs in the control population (<186 µg/mL, 186-211 µg/mL, 212-247 µg/mL, ≥248 µg/mL). Logistic regression models were used to estimate ORs of VTE with 95% CIs according to quartiles of C1INH adjusted for the matching factors, with the addition of BMI, CRP, and cancer as adjustment variables in a second model. The lowest quartile of C1INH was used as the reference group. *P* values for linear trends across increasing quartiles of C1INH were estimated. Separate analyses were additionally conducted with unprovoked and provoked VTE, DVT, and PE as outcomes, respectively. In these analyses, those with concomitant PE and DVT were regarded as PE. We also performed analyses restricted to individuals without cancer and sex-stratified analyses. The continuous association between C1INH levels and VTE was visualized in a spline plot. This plot was produced using the "gam" package in R (version 4.2.2) using smoothing splines with 3° of freedom. For these analyses, C1INH levels were transformed to follow a perfect normal distribution with a mean of 0 and a standard deviation of 1. Because of the long follow-up time (≥12 years for many individuals) in the source cohort, the results based on baseline C1INH measurements could be influenced by regression dilution bias. To address this, we performed analyses that restricted the maximum follow-up time from blood sampling to the VTE events, whereas keeping all controls in the analyses. The logistic regression analyses on time restrictions were set to require at least 10 VTE events, and ORs were generated at every time point a new VTE event occurred and plotted as a function of this maximum time.

2.4 | Step 3 – Genetic regulation of C1INH

Whole exome sequencing at high coverage (≈100×) was carried out in a random subset of the nested case-control study population (353 patients with VTE and 354 control subjects) by the use of the Agilent SureSelect 50Mb capture kit, as previously described [36,37]. We

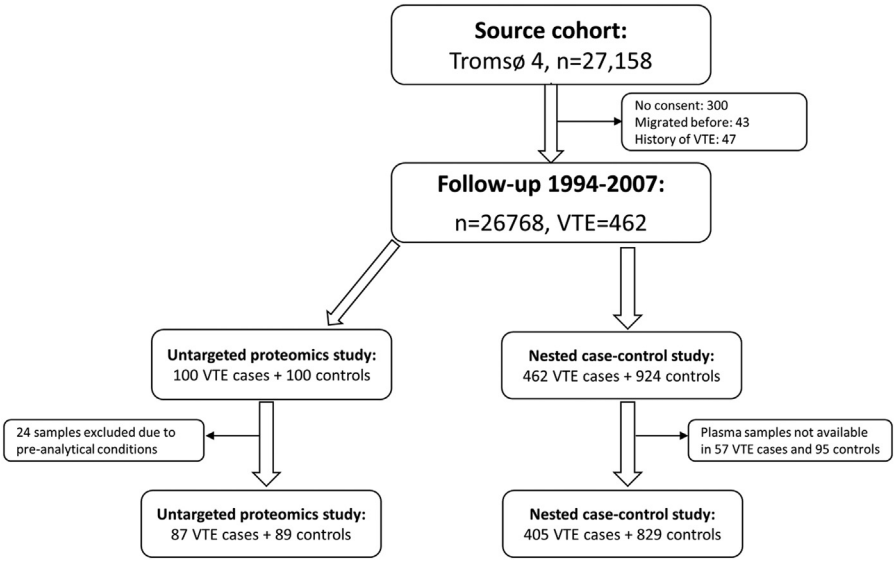

FIGURE 1 Overview of the source cohort, proteomics study and nested case-control study.

applied a pQTL analysis to identify genetic variants associated with the regulation of C1INH plasma levels. For the description of the pQTL analysis, please see Supplementary Material. The results of the pQTL analyses are presented in a Manhattan plot.

2.5 | Step 4 – Effect of C1INH on plasma-based thrombin and plasmin generation

2.5.1 | Thrombin generation assay

Thrombin generation was measured in citrate-anticoagulated plasma using calibrated automated thrombography (CAT, Fluoroscan Ascent, Thermo Fisher Scientific) as described previously [38]. In brief, exogenous human purified C1INH (Athens Research and Technology) was added to normal pooled human plasma (Innovative Research) at concentrations of 0, 100, 200, and 400 μg/mL, and incubated at room temperature for 5 minutes. Twenty μL of either silica (5 μM, Kontact, Thermo Fisher Scientific), lipidated tissue factor (TF) (5 pM or 1 pM, Thrombinoscope BV) or calibrator (Thrombinoscope BV) was added to 80 μL of plasma in the presence of 4 μM phospholipid and in the presence or absence of the FXIIa inhibitor corn trypsin inhibitor (CTI, 50 μg/mL, Molecular Innovations) and incubated at 37°C for 10 minutes. Reactions were initiated by automatic dispensing of 20 μL of FluCa solution (Thrombinoscope BV).

2.5.2 | Modified activated partial thromboplastin time assay and prothrombin time assay

Exogenous purified human C1INH was added to pooled normal human plasma at concentrations of 0, 100, 200, and 400 μg/mL, and incubated at room temperature for 5 minutes. For measurements of activated partial thromboplastin time (aPTT), 25 μL of TriniCLOT aPTT S reagent (Stago, NJ) was added to 25 μL of C1INH supplemented plasma and incubated at 37°C for 5 minutes. Coagulation was initiated by the addition of 25 μL of TriniCLOT aPTT S $CaCl_2$ reagent (Stago, NJ) and the clotting time of plasma was determined on a hemostasis analyzer (STart 4, Stago, NJ). For the measurement of prothrombin time, 100 μL of Thromboplastin-D reagent (Pacific Hemostasis, CA) was added to 50 μL of C1INH supplemented plasma and the clotting time of plasma determined on a hemostasis analyzer (STart 4, Stago, NJ).

2.5.3 | Plasmin generation assay

Plasmin generation was measured in 2:1 (plasma:buffer) diluted pooled normal human plasma, as previously described [39]. Human purified C1INH was added to plasma at concentrations of 100, 200, and 400 μg/mL, and compared with vehicle control, and incubated at room temperature for 5 minutes. Ten μL of lipidated TF (0.5 pM Innovin, Siemens Healthcare, Germany, 4 uM phospholipid) or α_2-macroglobulin-plasmin complex calibrator (kindly provided by Synapse Research Institute, Netherlands) was added to 40 μL of diluted plasma and incubated at 37 °C for 10 minutes. Reactions were initiated by automatic dispensing of 10 μL of substrate (0.5 mM Boc-Glu-Lys-Lys-AMC, Bachem, Switzerland, 16.6 mM $CaCl_2$). Data were analyzed using Thrombinoscope Analysis software (version 5.0.0.742, Thrombinoscope BV).

3 | RESULTS

3.1 | Untargeted proteomics case-control study

The baseline characteristics of the participants in the untargeted proteomics study are described in Supplementary Table S1a. The association between increasing quartiles (Q) of C1INH and the risk of VTE is shown in Figure 2. The OR for Q4 versus Q1 was 0.29 (95% CI:

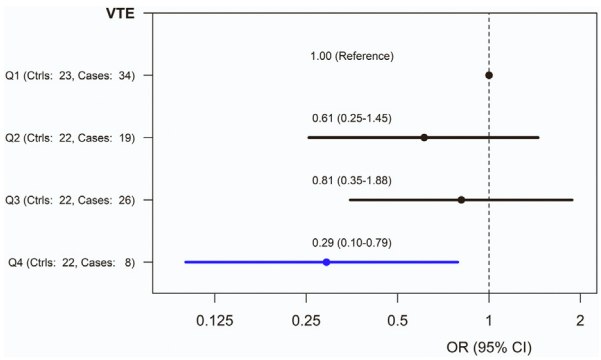

FIGURE 2 Association between plasma levels of C1-inhibitor (C1INH) and venous thromboembolism (VTE) in the untargeted proteomics study. Odds ratios (OR) of VTE with 95% confidence intervals (CI) across increasing quartiles (Q) of C1INH. ORs are adjusted for age, sex, body mass index and cancer.

0.10-0.79), demonstrating an inverse association between plasma C1INH levels and VTE risk.

3.2 | Association between C1INH and VTE in a nested case-control study

The distribution of baseline characteristics across quartiles of plasma C1INH levels in this study is shown in Table 1, whereas the baseline characteristics according to case and control status are given in Supplementary Table S1b. No major differences in baseline characteristics were found across the quartiles of C1INH. The characteristics of patients with VTE assessed at the time of the VTE event are shown in Table 2. The mean age at the time of VTE was 67 years, and most of the events were DVTs (62.5%) and provoked VTEs (58.3%). An overview of those included in the study and those excluded because of missing blood samples is provided in Supplementary Table S2.

TABLE 2 Characteristics of VTE events in the nested case-control study (n = 405).

Characteristics	Mean ± SD or n (%)
Age at VTE, y (±SD)	67 ± 14
Sex, % men	48.1 (195)
Deep vein thrombosis, % (n)	62.5 (253)
Pulmonary embolism, % (n)	37.5 (152)
Unprovoked VTE, % (n)	41.7 (169)
Provoked VTE, % (n)	58.3 (236)
Surgery/trauma, % (n)	22.5 (91)
Cancer, % (n)	20.7 (84)
Immobilization, % (n)	18.3 (74)
Acute medical condition, % (n)	16.0 (65)
Other factors, % (n)	4.2 (17)

The ORs for VTE, DVT, and PE across quartiles of plasma C1INH levels are shown in Figure 3. The risk of VTE was lower in the highest quartile of C1INH, with OR for Q4 versus Q1 of 0.66 (95% CI: 0.47-0.93) in analysis adjusted for age and sex. Further adjustment for BMI, plasma CRP levels, and cancer had a negligible impact on the risk estimates (OR: 0.68, 95% CI: 0.49-0.96). In subgroup analyses, the OR for Q4 vs. Q1 was 0.60 (95% CI: 0.39-0.89) for DVT and 0.85 (95% CI: 0.52-1.38) for PE in the fully adjusted model. The risk estimates for unprovoked and provoked VTE were in the same size and direction as the estimates for overall VTE (Figure 3), and analyses restricted to individuals without cancer showed similar results (Supplementary Figure S1). In sex-specific analyses, the association appeared to be somewhat more pronounced in women than in men, but the confidence intervals overlapped, indicating no significant difference between the sexes (Supplementary Table S3). The generalized additive regression plot showed a linear relationship

TABLE 1 Baseline characteristics of controls across quartiles (Q) of C1 inhibitor plasma levels in the nested case-control study.

Characteristics	Q1 (81-185 μg/mL)	Q2 (186-211 μg/mL)	Q3 (212-247 μg/mL)	Q4 (248-694 μg/mL)
N	207	207	208	208
Age, y (±SD)	60 ± 14	61 ± 14	61 ± 14	60 ± 14
Sex, % men (n)	42.0 (87)	42.5 (88)	53.6 (111)	46.6 (97)
BMI, kg/m^2	26.4 ± 4.1	25.9 ± 3.8	25.9 ± 4.1	25.9 ± 4.3
Smoking, % (n)	30.4 (63)	28.5 (59)	32.9 (68)	36.5 (76)
hsCRP, mg/L (±SD)	1.7 ± 1.5	1.4 ± 1.3	1.7 ± 1.4	1.5 ± 1.3
WBC, 10^9/L (±SD)	7.0 ± 1.7	6.9 ± 1.8	6.9 ± 1.9	7.0 ± 1.7
CVD, % (n)	17.9 (37)	14.5 (30)	15.0 (31)	15.4 (32)
Cancer, % (n)	4.3 (9)	2.4 (5)	4.3 (9)	3.4 (7)
Diabetes, % (n)	5.3 (11)	3.9 (8)	2.9 (6)	3.4 (7)

BMI, body mass index; CVD, cardiovascular disease (myocardial infarction, angina and stroke), hsCRP, high-sensitive C-reactive protein; WBC, white blood count.

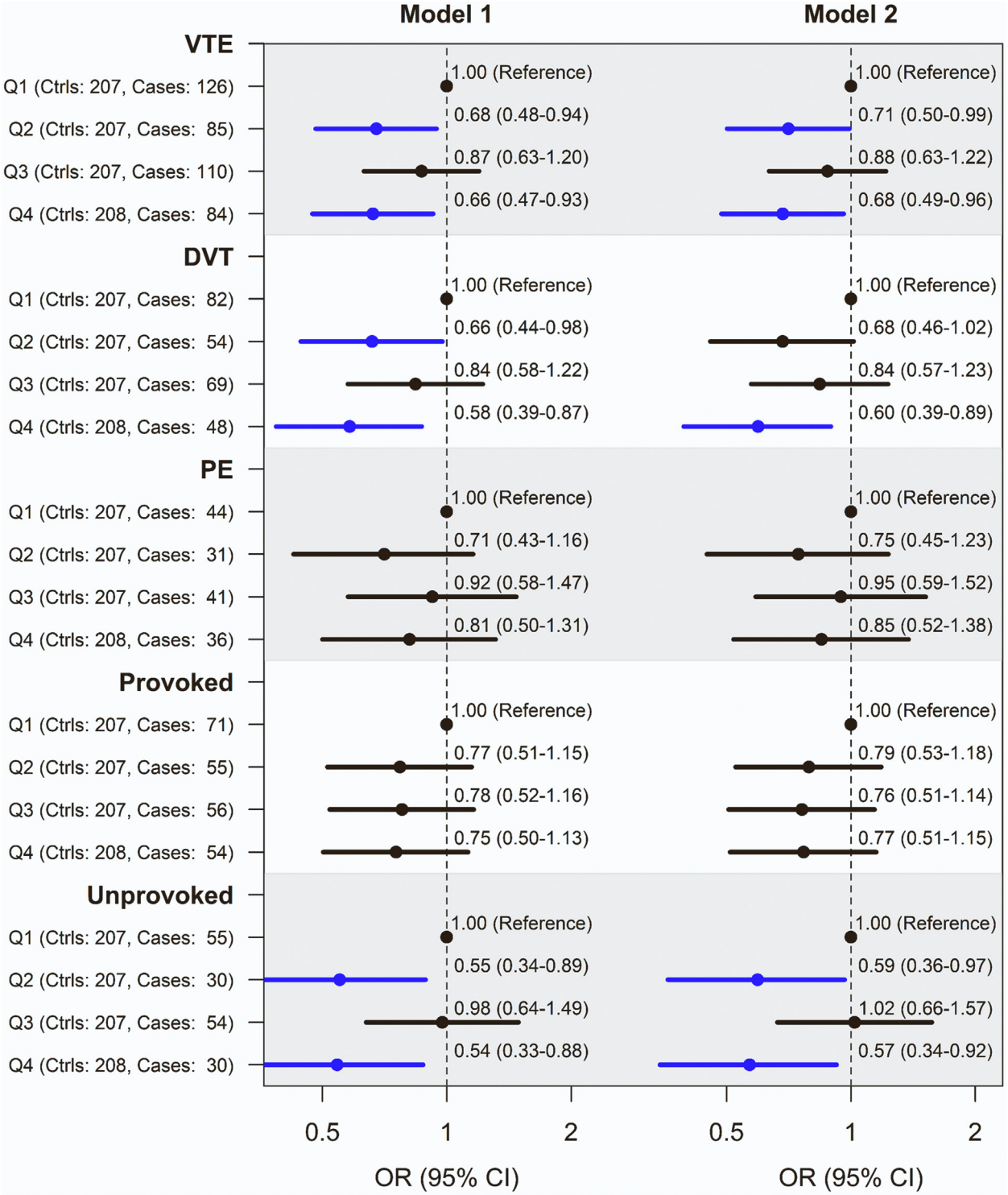

FIGURE 3 Association between plasma levels of C1-inhibitor (C1INH) and venous thromboembolism (VTE) in the nested case-control study. Odds ratios (OR) of VTE with 95% confidence intervals (CI) across increasing quartiles (Q) of C1INH. Model 1 (left) is adjusted for age and sex. Model 2 (right) is adjusted for age, sex, body mass index, C-reactive protein and cancer. DVT, deep vein thrombosis; PE, pulmonary embolism. Quartile cut-offs for C1INH are Q1: <186, Q2: 186-211, Q3: 212-247, Q4: ≥248 μg/mL.

between increasing levels of C1INH and decreasing OR of VTE (Supplementary Figure S2).

To consider the possibility of regression dilution bias with an underestimation of the true associations, we estimated ORs (highest vs. lowest quartile of C1INH) according to the time between blood sampling and VTE (Figure 4). The regression dilution plot revealed that the OR for VTE (Q4 vs. Q1) was essentially unchanged over time since blood sampling, indicating

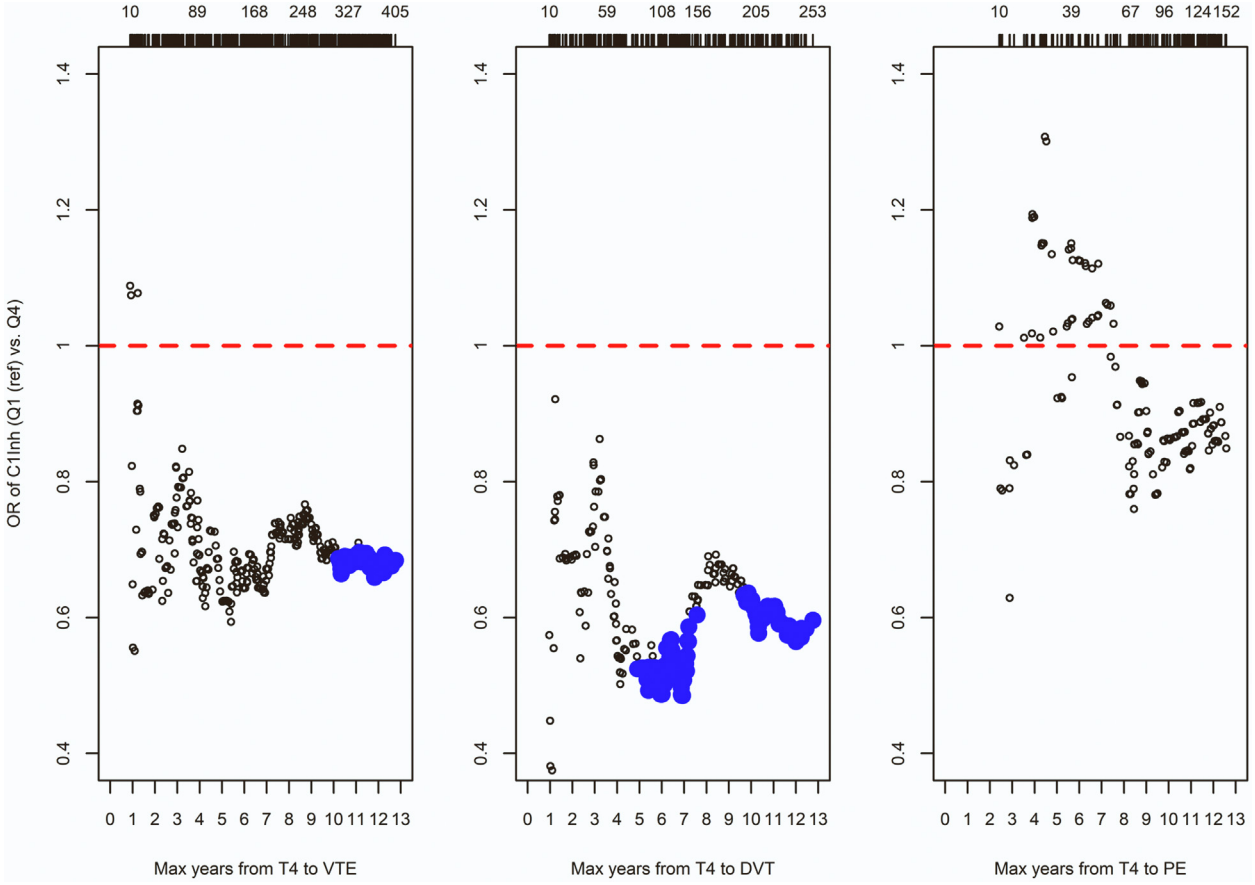

FIGURE 4 Longitudinal association between plasma C1-inhibitor (C1INH) and venous thromboembolism (VTE) risk in the nested case-control study. Plots of estimated odds ratios (OR) of VTE (left panel), deep vein thrombosis (DVT, middle panel) and pulmonary embolism (PE, right panel) as a function of maximum time from blood sampling in Tromsø 4 (1994-1995) to events in analyses adjusted for age, sex, body mass index, C-reactive protein and cancer. Subjects with plasma C1 inhibitor (C1INH) in the highest quartile (Q4: ≥248 μg/mL) were compared to those with C1INH levels in the lowest quartile (Q1: <186 μg/mL, reference category). The number of VTE, DVT and PE events are depicted above the plot. The blue, solid circles indicate ORs with *P* values <0.05.

minor or no regression dilution bias (Figure 4 and Supplementary Figure S3).

3.3 | Genetic regulation of C1INH

The result of the pQTL analysis is shown in Figure 5. No single-nucleotide polymorphism was significantly associated with plasma C1INH levels, either in the genome-wide or the cis analysis.

3.4 | Effect of exogenous C1INH on intrinsic pathway-initiated thrombin generation

To investigate the anticoagulant activity of C1INH, the effects of exogenous purified human C1INH on plasma-based thrombin generation were assessed by CAT. Exogenous purified human C1INH (0, 100, 200, 400 μg/mL) was added to normal pooled plasma, resulting in final concentrations (190, 290, 390, and 590 μg/mL) that largely covered the range of C1INH levels measured in the nested case-control study. The addition of increasing concentrations of exogenous C1INH resulted in reduced thrombin generation triggered using silica (Figure 6A). The addition of exogenous human purified C1INH to normal human plasma resulted in significant dose-dependent prolongation of lag time (Figure 6B) and time to peak (Figure 6C), along with reductions in velocity (Figure 6D), peak (Figure 6E), and endogenous thrombin potential (Figure 6F). The addition of exogenous human purified C1INH at 400 μg/mL, roughly twice the amount present in normal human plasma, resulted in a marked increase in lag time (83%), increase in time to peak (94%), reduction in velocity (83%), reduction in peak (63%), and reduction in endogenous thrombin potential (25%).

Consistent with the observed effect of C1INH on silica-initiated thrombin generation, the addition of exogenous human C1INH to normal human plasma resulted in a dose-dependent prolongation of clotting time in a modified activated partial thromboplastin time assay at all concentrations evaluated (Supplementary Figure S4A).

3.5 | Effect of exogenous C1INH on extrinsic pathway-initiated thrombin generation

To further investigate the anticoagulant activity of C1INH, thrombin generation was assessed using the extrinsic pathway trigger TF

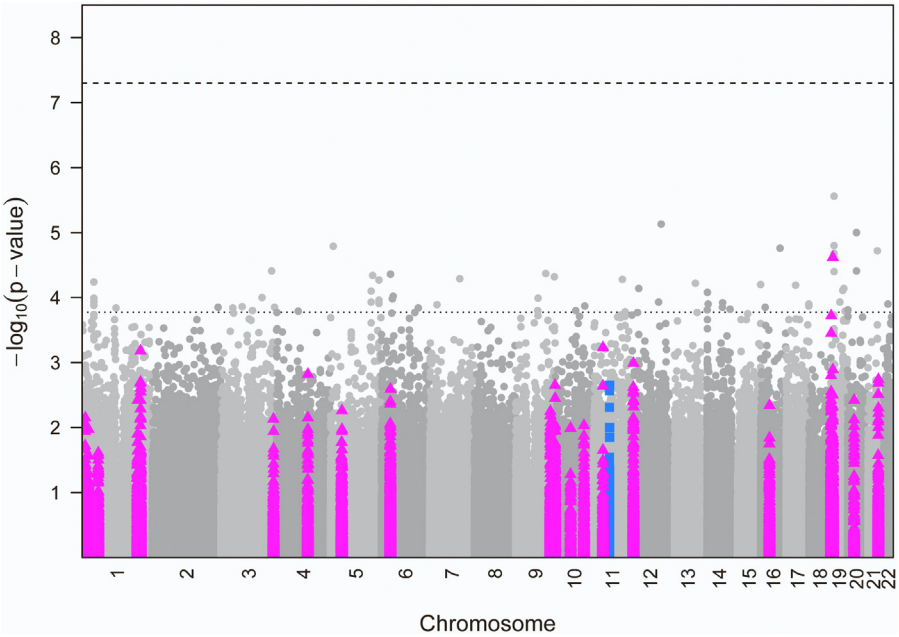

FIGURE 5 Analysis of genetic determinants of plasma C1-inhibitor (C1INH). Manhattan plot of protein quantitative trait loci (pQTL) analysis (GRCh37/hg19 was used as reference human genome). The upper, dashed line indicates the $5x10^{-8}$ P value significance threshold. The purple triangles indicate complement-related genes, and the blue squares indicate the *SERPING1* gene.

(Supplementary Figure S5). Thrombin generation initiated with a low concentration of TF in the presence of the FXIIa inhibitor CTI was previously shown to occur in a FXI-dependent manner [40]. In the presence of CTI, the addition of exogenous human purified C1INH to normal human plasma had no significant effect on thrombin generation initiated with 5 pM or 1 pM TF (Supplementary Figure S5). Accordingly, no significant effect of exogenous human C1INH was observed in a modified prothrombin time assay (Supplementary Figure S5).

3.6 | Effect of exogenous C1INH on plasmin generation

C1INH also has the antifibrinolytic activity through its ability to inhibit plasmin and tissue plasminogen activator [41,42]. The effect of exogenous C1INH on fibrinolysis was assessed using a plasma-based calibrated plasmin generation assay. No effect of exogenous C1INH, at concentrations that significantly inhibited silica-initiated thrombin generation, was detected (Supplementary Figure S6).

4 | DISCUSSION

In a nested case-control study of 405 patients with VTE and 829 controls derived from the general population, we showed that plasma C1INH levels within the upper normal range were associated with lower VTE risk. Participants with C1INH levels in the highest quartile had a 36% lower risk of VTE after adjustment for BMI, CRP, and cancer. The ORs for VTE by C1INH remained essentially unchanged over time between blood sampling and VTE occurrence, suggesting a minor regression dilution because of intra-individual fluctuation in C1INH levels over time. The stable association between C1INH levels and VTE over time suggests that C1INH could serve as a robust short- and long-term biomarker of VTE risk.

From an etiological point of view, a clear temporal sequence between exposure (measured plasma C1INH level) and outcome (VTE), such as in our nested case-control study, is a prerequisite to recognize plasma C1INH as a risk factor for VTE. Our study design minimized the risk of reverse causation [43], but observational studies may still be prone to confounding [44]. Even though the association between C1INH and VTE risk was only modestly influenced by multivariable adjustments for potential confounders, residual confounding cannot be ruled out [45] and makes causal inference challenging. Mendelian randomization (MR) analysis is a method designed to unravel causal relationships between exposure and outcome in observational studies built on the fact that gene variants robustly associated with modifiable exposure variables, such as plasma C1INH levels, are fixed at conception and follow Mendel's Law of inheritance [46,47]. However, in our pQTL analysis and literature search, we did not identify gene variants associated with plasma C1INH levels, and therefore, we could not use MR analyses to assess whether the association between plasma C1INH levels and VTE risk could be causal.

To investigate how normal variation in plasma levels of C1INH could influence the hemostatic mechanisms involved in thrombosis formation, we conducted a series of *ex vivo* investigations. The addition of exogenous C1INH at physiologically relevant concentrations resulted in a selective and dose-dependent reduction in silica-initiated thrombin generation, but not in TF-initiated thrombin generation or plasmin generation. In agreement with previous reports [48–50], our data suggest that C1INH functions as an important inhibitor of the intrinsic pathway of coagulation. We speculate

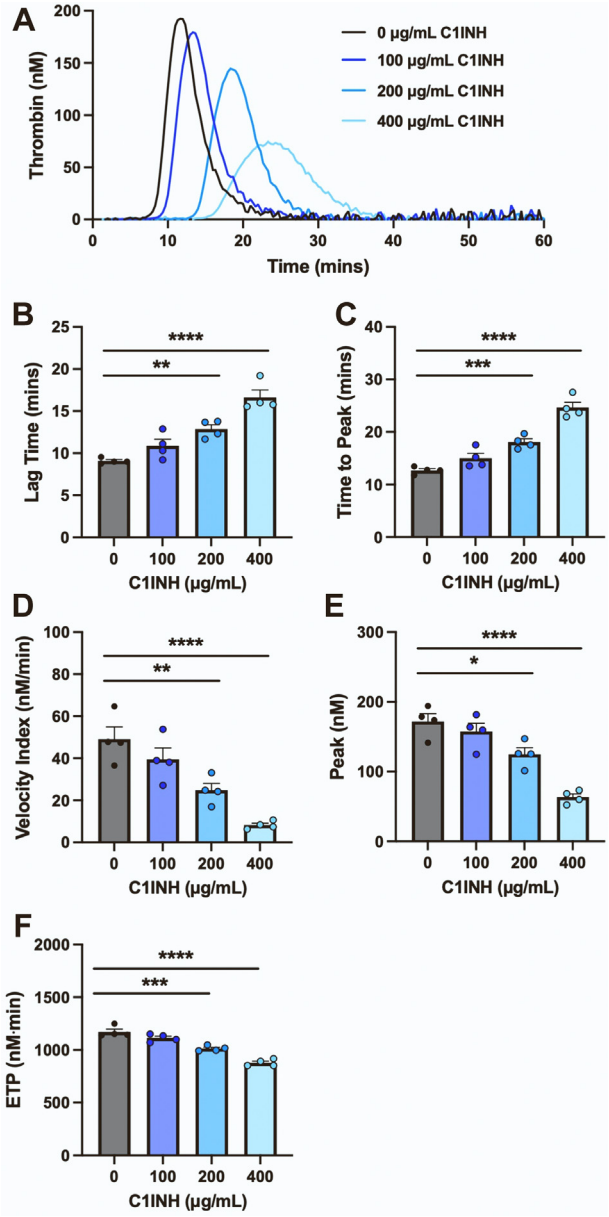

FIGURE 6 Effect of exogenous C1-inhibitor (C1INH) on intrinsic pathway-initiated thrombin generation. (A) Representative curves of silica-initiated thrombin generation conducted in normal pooled human plasma in the presence of increasing concentrations of exogenous human purified C1INH. Quantification of (B) lagtime, (C) time to peak, (D) velocity index, (E) peak and (F) endogenous thrombin potential (ETP). Data from n=4 independent experiments represented as individual values with mean and standard error of the mean. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, one-way ANOVA with post-hoc Bonferroni.

that the intrinsic pathway-mediated anticoagulant activity of C1INH likely contributes to the inverse association between plasma C1INH levels and risk of VTE.

It is important to note that the observed effects of C1INH supplementation on intrinsic pathway-initiated thrombin generation do not directly explain the inverse association between plasma C1INH and VTE risk. However, we recently reported that congenital C1INH deficiency in humans results in increased intrinsic pathway-initiated coagulation and is associated with an increased risk of VTE [27,28,51]. Several studies have shown that increased plasma thrombin generation is associated with incident [52,53] and recurrent [54,55] VTE. Findings from patients with congenital FXI deficiency provide further insights, since FXIa is a target for C1INH. FXI deficiency has been associated with a reduced incidence of VTE [17,56], and plasmas from FXI-deficient patients show reduced thrombin generation when initiated by a low dose of TF [57]. Moreover, inhibition of FXI reduced the risk of surgery-related VTE in recent phase 2 clinical trials [18–21].

Preclinical studies provide additional evidence indicating that the C1INH targets FXIIa and FXIa contribute to the pathogenesis of VTE. Loss of plasma FXII or FXI in mice, achieved either by gene deletion or transient gene knockdown, significantly inhibits venous thrombus formation [15, 58–60]. Transient knockdown of the gene encoding the C1INH target plasma kallikrein also reduces venous thrombus formation [58]. Furthermore, exogenous administration of C1INH has been shown to reduce arterial thrombosis in a rabbit model [48]. Importantly, we recently demonstrated that C1INH deficiency in mice resulted in increased intrinsic pathway-initiated thrombin generation and venous thrombosis [51]. This phenotype complements the findings in humans with congenital C1INH deficiency [27,28,51].

The inhibitory function of C1INH on activation of the complement system may also contribute to the inverse association between C1INH levels and VTE risk. C1INH serves as the major endogenous inhibitor of factors that comprise the first component of complement, the complex of C1q with serine proteases C1r and C1s, that are essential for the activation of the classical pathway [61]. In addition, C1INH also inhibits the lectin pathway factors MASP-1 and 2 [62], which have the ability to cleave prothrombin to thrombin [26]. We recently reported that high plasma complement activation, assessed by circulating levels of the terminal complement complex, was associated with an increased risk of VTE [63]. Consistent with this finding, we recently reported that high plasma levels of mannose-binding lectin-associated protease 2 were associated with an increased risk of VTE [64]. Furthermore, elevated plasma complement components C3 and C5 levels are also associated with an increased risk of VTE [65,66]. These findings are consistent with preclinical evidence demonstrating reduced incidence and size of venous thrombus formation in complement C3 and C5 deficient mice [67].

It is possible that C1INH could serve as a useful biomarker for future VTE risks. Clinical assays for both C1INH antigen and C1INH activity have been developed and are used in the diagnosis of C1INH deficiency-associated hereditary angioedema [68]. In addition, human purified and recombinant C1INH preparations have been developed as treatments for patients with hereditary angioedema [69]. It is interesting to consider if human C1INH preparations could find additional utility as an intrinsic pathway-targeted anticoagulant in the setting of VTE. Further work is required to establish the clinical utility of these approaches.

Strengths of this study include the temporal sequence of exposure and outcome in a study recruited from the general adult population with validated VTE events, access to exome sequencing data, and

measured plasma C1INH levels in the same population, along with supportive data from *ex vivo* models. The study also has limitations. Blood samples were drawn in 1994-1995 and stored at −80 °C for up to 22 years. The long storage time could affect the plasma C1INH levels. However, plasma C1INH levels in our study population were similar to those in previous reports among healthy individuals and blood donors [70]. Additionally, as all samples were stored under the same conditions and for the same amount of time for cases and controls, the storage effect is assumed to be similar in the 2 groups, and any influence would therefore likely result in regression dilution (ie, underestimation of the true effect). Even though C1INH was assessed at only 1-time point (ie, at cohort inclusion), our regression dilution plot indicated that the association with VTE was stable over time. All observational studies are prone to bias and chance findings, and we cannot rule out that the observed association between C1INH and VTE is by chance. However, the fact that we confirmed our findings from the MS-based case-control study in a larger study with C1INH levels measured with a different method (immunoassay), together with our experimental studies supporting the role of C1INH for thrombin inhibition by the intrinsic coagulation system, suggests that our observations are not due to chance.

In conclusion, our studies showed that plasma C1INH levels were inversely associated with VTE risk. Experimental *ex vivo* studies showed that C1INH inhibits thrombin generation triggered by the intrinsic coagulation system. Further mechanistic studies are needed to support a causative role of increased C1INH levels for the protection against VTE.

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AUTHOR CONTRIBUTIONS

Conception and design: J.B.H., S.K.B., S.P.G., N.M.; Data collection: S.P.G., O.S., S.K.B., S.B.J., A.S.W., T.E.M., T.U., T.E., J.B.H.; Statistical analysis: S.P.G., K.H., V.M.M., S.K.B.; Interpretation of data: S.P.G., O.S., K.H., S.K.B., V.M.M., T.E.M., A.S.W., N.M., J.B.H.; Draft of manuscript: S.P.G., J.B.H., S.K.B.; Review and approval of the final version: All authors.

DECLARATION OF COMPETING INTERESTS

There are no competing interests to disclose.

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REFERENCES

[1] Naess IA, Christiansen SC, Romundstad P, Cannegieter SC, Rosendaal FR, Hammerstrom J. Incidence and mortality of venous thrombosis: a population-based study. *J Thromb Haemost*. 2007;5:692–9.
[2] Arshad N, Isaksen T, Hansen JB, Braekkan SK. Time trends in incidence rates of venous thromboembolism in a large cohort recruited from the general population. *Eur J Epidemiol*. 2017;32:299–305.
[3] Winter MP, Schernthaner GH, Lang IM. Chronic complications of venous thromboembolism. *J Thromb Haemost*. 2017;15:1531–40.
[4] Schulman S, Lindmarker P, Holmstrom M, Larfars G, Carlsson A, Nicol P, Svensson E, Ljungberg B, Viering S, Nordlander S, Leijd B, Jahed K, Hjorth M, Linder O, Beckman M. Post-thrombotic syndrome, recurrence, and death 10 years after the first episode of venous thromboembolism treated with warfarin for 6 weeks or 6 months. *J Thromb Haemost*. 2006;4:734–42.
[5] Klok FA, van der Hulle T, den Exter PL, Lankeit M, Huisman MV, Konstantinides S. The post-PE syndrome: a new concept for chronic complications of pulmonary embolism. *Blood Rev*. 2014;28:221–6.
[6] Jorgensen H, Horvath-Puho E, Laugesen K, Braekkan S, Hansen JB, Sorensen HT. Risk of a permanent work-related disability pension after incident venous thromboembolism in Denmark: a population-based cohort study. *PLoS Med*. 2021;18:e1003770.
[7] Cushman M. Epidemiology and risk factors for venous thrombosis. *Semin Hematol*. 2007;44:62–9.
[8] Lijfering WM, Rosendaal FR, Cannegieter SC. Risk factors for venous thrombosis - current understanding from an epidemiological point of view. *Br J Haematol*. 2010;149:824–33.
[9] Kearon C, Ageno W, Cannegieter SC, Cosmi B, Geersing GJ, Kyrle PA. Subcommittees on Control of Anticoagulation and Predictive and Diagnostic Variables in Thrombotic Disease. Categorization of patients as having provoked or unprovoked venous thromboembolism: guidance from the SSC of ISTH. *J Thromb Haemost*. 2016;14:1480–3. https://doi.org/10.1111/jth.13336
[10] Christensen DM, Strange JE, Phelps M, Schjerning AM, Sehested TSG, Gerds T, Gislason G. Age- and sex-specific trends in the incidence of myocardial infarction in Denmark, 2005 to 2021. *Atherosclerosis*. 2022;346:63–7.
[11] Sulo G, Igland J, Vollset SE, Ebbing M, Egeland GM, Ariansen I, Tell GS. Trends in incident acute myocardial infarction in Norway: an updated analysis to 2014 using national data from the CVDNOR project. *Eur J Prev Cardiol*. 2018;25:1031–9.
[12] Munster AM, Rasmussen TB, Falstie-Jensen AM, Harboe L, Stynes G, Dybro L, Hansen ML, Brandes A, Grove EL, Johnsen SP. A changing landscape: temporal trends in incidence and characteristics of patients hospitalized with venous thromboembolism 2006-2015. *Thromb Res*. 2019;176:46–53.
[13] Ghanima W, Brodin E, Schultze A, Shepherd L, Lambrelli D, Ulvestad M, Ramagopalan S, Halvorsen S. Incidence and prevalence of venous thromboembolism in Norway 2010-2017. *Thromb Res*. 2020;195:165–8.
[14] Wandell P, Forslund T, Danin Mankowitz H, Ugarph-Morawski A, Eliasson S, Braunschweig F, Holmstrom M. Venous thromboembolism 2011-2018 in Stockholm: a demographic study. *J Thromb Thrombolysis*. 2019;48:668–73.

[15] Grover SP, Mackman N. Intrinsic pathway of coagulation and thrombosis. *Arterioscler Thromb Vasc Biol*. 2019;39:331–8.
[16] Meijers JC, Tekelenburg WL, Bouma BN, Bertina RM, Rosendaal FR. High levels of coagulation factor XI as a risk factor for venous thrombosis. *N Engl J Med*. 2000;342:696–701.
[17] Salomon O, Steinberg DM, Zucker M, Varon D, Zivelin A, Seligsohn U. Patients with severe factor XI deficiency have a reduced incidence of deep-vein thrombosis. *Thromb Haemost*. 2011;105:269–73.
[18] Buller HR, Bethune C, Bhanot S, Gailani D, Monia BP, Raskob GE, Segers A, Verhamme P, Weitz JI, Investigators F-AT. Factor XI antisense oligonucleotide for prevention of venous thrombosis. *N Engl J Med*. 2015;372:232–40.
[19] Weitz JI, Bauersachs R, Becker B, Berkowitz SD, Freitas MCS, Lassen MR, Metzig C, Raskob GE. Effect of osocimab in preventing venous thromboembolism among patients undergoing knee arthroplasty: the FOXTROT randomized clinical trial. *Jama*. 2020;323:130–9.
[20] Verhamme P, Yi BA, Segers A, Salter J, Bloomfield D, Buller HR, Raskob GE, Weitz JI, Investigators A-T. Abelacimab for prevention of venous thromboembolism. *N Engl J Med*. 2021;385:609–17.
[21] Weitz JI, Strony J, Ageno W, Gailani D, Hylek EM, Lassen MR, Mahaffey KW, Notani RS, Roberts R, Segers A, Raskob GE, Investigators A-T. Milvexian for the prevention of venous thromboembolism. *N Engl J Med*. 2021;385:2161–72.
[22] Zeerleder S. C1-inhibitor: more than a serine protease inhibitor. *Semin Thromb Hemost*. 2011;37:362–74.
[23] Wuillemin WA, Minnema M, Meijers JC, Roem D, Eerenberg AJ, Nuijens JH, ten Cate H, Hack CE. Inactivation of factor XIa in human plasma assessed by measuring factor XIa-protease inhibitor complexes: major role for C1-inhibitor. *Blood*. 1995;85:1517–26.
[24] de Agostini A, Lijnen HR, Pixley RA, Colman RW, Schapira M. Inactivation of factor XII active fragment in normal plasma. Predominant role of C-1-inhibitor. *J Clin Invest*. 1984;73:1542–9.
[25] Davis AE, Mejia P, Lu F. Biological activities of C1 inhibitor. *Mol Immunol*. 2008;45:4057–63.
[26] Wong NK, Kojima M, Dobo J, Ambrus G, Sim RB. Activities of the MBL-associated serine proteases (MASPs) and their regulation by natural inhibitors. *Mol Immunol*. 1999;36:853–61.
[27] Grover SP, Sundler Bjorkman L, Egesten A, Moll S, Mackman N. Hereditary angioedema is associated with an increased risk of venous thromboembolism. *J Thromb Haemost*. 2022;20:2703–6.
[28] Sundler Bjorkman L, Persson B, Aronsson D, Skattum L, Nordenfelt P, Egesten A. Comorbidities in hereditary angioedema-a population-based cohort study. *Clin Transl Allergy*. 2022;12:e12135.
[29] Schreiber AD, Kaplan AP, Austen KF. Inhibition by C1INH of Hagemann factor fragment activation of coagulation, fibrinolysis, and kinin generation. *J Clin Invest*. 1973;52:1402–9.
[30] Tarandovskiy ID, Rajabi AA, Karnaukhova E, Buehler PW. Contradictory to its effects on thrombin, C1-inhibitor reduces plasmin generation in the presence of thrombomodulin. *J Thromb Thromb*. 2019;48:81–7.
[31] Meltzer ME, Lisman T, de Groot PG, Meijers JC, le Cessie S, Doggen CJ, Rosendaal FR. Venous thrombosis risk associated with plasma hypofibrinolysis is explained by elevated plasma levels of TAFI and PAI-1. *Blood*. 2010;116:113–21.
[32] Jensen SB, Hindberg K, Solomon T, Smith EN, Lapek JD, Gonzalez DJ, Latysheva N, Frazer KA, Braekkan SK, Hansen JB. Discovery of novel plasma biomarkers for future incident venous thromboembolism by untargeted synchronous precursor selection mass spectrometry proteomics. *J Thromb Haemost*. 2018;16:1763–74.
[33] Jacobsen BK, Eggen AE, Mathiesen EB, Wilsgaard T, Njolstad I. Cohort profile: the tromso study. *Int J Epidemiol*. 2012;41:961–7.
[34] Braekkan SK, Mathiesen EB, Njolstad I, Wilsgaard T, Stormer J, Hansen JB. Mean platelet volume is a risk factor for venous thromboembolism: the Tromso Study, Tromso, Norway. *J Thromb Haemost*. 2010;8:157–62.
[35] Hansen ES, Hindberg K, Latysheva N, Aukrust P, Ueland T, Hansen JB, Braekkan SK, Morelli VM, Consortium I. Plasma levels of growth differentiation factor 15 are associated with future risk of venous thromboembolism. *Blood*. 2020;136:1863–70.
[36] Carson AR, Smith EN, Matsui H, Braekkan SK, Jepsen K, Hansen JB, Frazer KA. Effective filtering strategies to improve data quality from population-based whole exome sequencing studies. *BMC Bioinformatics*. 2014;15:125.
[37] Solomon T, Lapek JD, Jr., Jensen SB, Greenwald WW, Hindberg K, Matsui H, Latysheva N, Braekken SK, Gonzalez DJ, Frazer KA, Smith EN, Hansen JB. Identification of common and rare genetic variation associated with plasma protein levels using whole-exome sequencing and mass spectrometry. *Circ Genom Precis Med*. 2018;11:e002170.
[38] Machlus KR, Colby EA, Wu JR, Koch GG, Key NS, Wolberg AS. Effects of tissue factor, thrombomodulin and elevated clotting factor levels on thrombin generation in the calibrated automated thrombogram. *Thromb Haemost*. 2009;102:936–44.
[39] Miszta A, Ahmadzia HK, Luban NLC, Li S, Guo D, Holle LA, Berger JS, James AH, Gobburu JVS, van den Anker J, de Laat B, Wolberg AS. Application of a plasmin generation assay to define pharmacodynamic effects of tranexamic acid in women undergoing cesarean delivery. *J Thromb Haemost*. 2021;19:221–32.
[40] Kravtsov DV, Matafonov A, Tucker EI, Sun MF, Walsh PN, Gruber A, Gailani D. Factor XI contributes to thrombin generation in the absence of factor XII. *Blood*. 2009;114:452–8.
[41] Ratnoff OD, Pensky J, Ogston D, Naff GB. The inhibition of plasmin, plasma kallikrein, plasma permeability factor, and the C'1r subcomponent of the first component of complement by serum C'1 esterase inhibitor. *J Exp Med*. 1969;129:315–31.
[42] Huisman LG, van Griensven JM, Kluft C. On the role of C1-inhibitor as inhibitor of tissue-type plasminogen activator in human plasma. *Thromb Haemost*. 1995;73:466–71.
[43] Clarke R, Shipley M, Lewington S, Youngman L, Collins R, Marmot M, Peto R. Underestimation of risk associations due to regression dilution in long-term follow-up of prospective studies. *Am J Epidemiol*. 1999;150:341–53.
[44] Smith GD, Ebrahim S. Data dredging, bias, or confounding. *Bmj*. 2002;325:1437–8.
[45] Fewell Z, Davey Smith G, Sterne JA. The impact of residual and unmeasured confounding in epidemiologic studies: a simulation study. *Am J Epidemiol*. 2007;166:646–55.
[46] Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. *Hum Mol Genet*. 2014;23:R89–98.
[47] Zheng J, Baird D, Borges MC, Bowden J, Hemani G, Haycock P, Evans DM, Smith GD. Recent developments in mendelian randomization studies. *Curr Epidemiol Rep*. 2017;4:330–45.
[48] Schurmann D, Herzog E, Raquet E, Nolte MW, May F, Muller-Cohrs J, Bjorkqvist J, Dickneite G, Pragst I. C1-esterase inhibitor treatment: preclinical safety aspects on the potential prothrombotic risk. *Thromb Haemost*. 2014;112:960–71.
[49] Csuka D, Veszeli N, Imreh E, Zotter Z, Skopal J, Prohaszka Z, Varga L, Farkas H. Comprehensive study into the activation of the plasma enzyme systems during attacks of hereditary angioedema due to C1-inhibitor deficiency. *Orphanet J Rare Dis*. 2015;10:132.

[50] Bork K, Witzke G. Shortened activated partial thromboplastin time may help in diagnosing hereditary and acquired angioedema. *Int Arch Allergy Immunol*. 2016;170:101–7.
[51] Grover SP, Kawano T, Wan J, Tanratana P, Polai Z, Shim YJ, et al. C1 inhibitor deficiency enhances contact pathway mediated activation of coagulation and venous thrombosis. *Blood*. 2023. https://doi.org/10.1182/blood.2022018849
[52] van Hylckama Vlieg A, Christiansen SC, Luddington R, Cannegieter SC, Rosendaal FR, Baglin TP. Elevated endogenous thrombin potential is associated with an increased risk of a first deep venous thrombosis but not with the risk of recurrence. *Br J Haematol*. 2007;138:769–74.
[53] Lutsey PL, Folsom AR, Heckbert SR, Cushman M. Peak thrombin generation and subsequent venous thromboembolism: the Longitudinal Investigation of Thromboembolism Etiology (LITE) study. *J Thromb Haemost*. 2009;7:1639–48.
[54] Hron G, Kollars M, Binder BR, Eichinger S, Kyrle PA. Identification of patients at low risk for recurrent venous thromboembolism by measuring thrombin generation. *Jama*. 2006;296:397–402.
[55] Eichinger S, Hron G, Kollars M, Kyrle PA. Prediction of recurrent venous thromboembolism by endogenous thrombin potential and D-dimer. *Clin Chem*. 2008;54:2042–8.
[56] Preis M, Hirsch J, Kotler A, Zoabi A, Stein N, Rennert G, Saliba W. Factor XI deficiency is associated with lower risk for cardiovascular and venous thromboembolism events. *Blood*. 2017;129:1210–5.
[57] Pike GN, Cumming AM, Hay CR, Bolton-Maggs PH, Burthem J. Sample conditions determine the ability of thrombin generation parameters to identify bleeding phenotype in FXI deficiency. *Blood*. 2015;126:397–405.
[58] Revenko AS, Gao D, Crosby JR, Bhattacharjee G, Zhao C, May C, Gailani D, Monia BP, MacLeod AR. Selective depletion of plasma prekallikrein or coagulation factor XII inhibits thrombosis in mice without increased risk of bleeding. *Blood*. 2011;118:5302–11.
[59] von Bruhl ML, Stark K, Steinhart A, Chandraratne S, Konrad I, Lorenz M, Khandoga A, Tirniceriu A, Coletti R, Kollnberger M, Byrne RA, Laitinen I, Walch A, Brill A, Pfeiler S, Manukyan D, Braun S, Lange P, Riegger J, Ware J, Eckart A, Haidari S, Rudelius M, Schulz C, Echtler K, Brinkmann V, Schwaiger M, Preissner KT, Wagner DD, Mackman N, Engelmann B, Massberg S. Monocytes, neutrophils, and platelets cooperate to initiate and propagate venous thrombosis in mice in vivo. *J Exp Med*. 2012;209:819–35.
[60] Grover SP, Olson TM, Cooley BC, Mackman N. Model-dependent contributions of FXII and FXI to venous thrombosis in mice. *J Thromb Haemost*. 2020;18:2899–909.
[61] Ratnoff OD, Lepow IH. Some properties of an esterase derived from preparations of the first component of complement. *J Exp Med*. 1957;106:327–43.
[62] Presanis JS, Hajela K, Ambrus G, Gal P, Sim RB. Differential substrate and inhibitor profiles for human MASP-1 and MASP-2. *Mol Immunol*. 2004;40:921–9.
[63] Hoiland , II, Liang RA, Braekkan SK, Pettersen K, Ludviksen JK, Latysheva N, Snir O, Ueland T, Hindberg K, Mollnes TE, Hansen JB. Complement activation assessed by the plasma terminal complement complex and future risk of venous thromboembolism. *J Thromb Haemost*. 2019;17:934–43.
[64] Damoah CE, Snir O, Hindberg K, Garred P, Ludviksen JK, Braekkan SK, Morelli VM, Mollnes TE, Hansen JB, Consortium I. High levels of complement activating enzyme masp-2 are associated with the risk of future incident venous thromboembolism. *Arterioscler Thromb Vasc Biol*. 2022;42:1186–97.
[65] Skjeflo EW, Braekkan SK, Ludviksen JK, Snir O, Hindberg K, Mollnes TE, et al. Elevated plasma concentration of complement factor C5 is associated with risk of future venous thromboembolism. *Blood*. 2021;138:2129–37.
[66] Norgaard I, Nielsen SF, Nordestgaard BG. Complement C3 and high risk of venous thromboembolism: 80517 individuals from the Copenhagen general population study. *Clin Chem*. 2016;62:525–34.
[67] Subramaniam S, Jurk K, Hobohm L, Jackel S, Saffarzadeh M, Schwierczek K, Wenzel P, Langer F, Reinhardt C, Ruf W. Distinct contributions of complement factors to platelet activation and fibrin formation in venous thrombus development. *Blood*. 2017;129:2291–302.
[68] Cicardi M, Aberer W, Banerji A, Bas M, Bernstein JA, Bork K, Caballero T, Farkas H, Grumach A, Kaplan AP, Riedl MA, Triggiani M, Zanichelli A, Zuraw B, Hutpo EAACI. Classification, diagnosis, and approach to treatment for angioedema: consensus report from the Hereditary Angioedema International Working Group. *Allergy*. 2014;69:602–16.
[69] Cicardi M, Zanichelli A. Replacement therapy with C1 esterase inhibitors for hereditary angioedema. *Drugs Today (Barc)*. 2010;46:867–74.
[70] Tange CE, Kaur A, Verma N, Hickey A, Grigoriadou S, Scott C, Kiani S, Steven R, Ponsford M, El-Shanawany T, Jolles S, Harding S, Parker AR. Quantification of human C1 esterase inhibitor protein using an automated turbidimetric immunoassay. *J Clin Lab Anal*. 2019;33:e22627.

SUPPLEMENTARY MATERIAL

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