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Plasma Concentrations of High Molecular Weight Kininogen and Prekallikrein and Venous Thromboembolism Incidence in the General Population

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Abstract

The kallikrein/kinin system, an intravascular biochemical pathway that includes several proteins involved in the contact activation system of coagulation, renin-angiotensin activation, and inflammation, may or may not play a role in venous thromboembolism (VTE) occurrence. Within a large prospective population-based study in the United States, we conducted a nested case-cohort study to test the hypothesis that higher plasma levels of high molecular weight kininogen (HK) or prekallikrein are associated with greater VTE incidence. We related baseline ELISA measures of HK and prekallikrein in 1993–95 to incidence VTE of the lower extremity (n=612) through 2015 (mean follow-up = 18 years). We found no evidence that plasma HK or prekallikrein was associated positively with incident VTE. HK, in fact, was associated inversely and significantly

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Authors' Contributions

Aaron R. Folsom had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. The contributions of the authors are as follows:

A. R. Folsom: Study conception and design, analyzed the data, acquisition of data, drafted the manuscript, and final approval of the manuscript.

W. Tang: Provided critical revisions to the manuscript, and final approval of the manuscript.

S. Basu: Study conception and design, provided critical revisions to the manuscript, and final approval of the manuscript.

J. R. Misialek: Analyzed the data, provided critical revisions to the manuscript, and final approval of the manuscript.

D. Couper: Analyzed the data, provided critical revisions to the manuscript, and final approval of the manuscript.

S. R. Heckbert: Provided critical revisions to the manuscript, and final approval of the manuscript.

M. Cushman: Study conception and design, acquisition of data, provided critical revisions to the manuscript, and final approval of the manuscript.

Conflicts of Interest

None.

with VTE in most proportional hazards regression models. For example, the hazard ratio of VTE per standard deviation higher HK concentration was 0.88 (95% confidence interval = 0.81, 0.97), after adjustment for several VTE risk factors. Our findings suggest that plasma levels of these factors do not determine the risk of VTE in the general population.

Keywords

high molecular weight kininogen; prekallikrein; prospective study; pulmonary embolus; venous thrombosis

Introduction

Aberrant hemostasis pathways play key roles in the etiology of deep vein thrombosis (DVT) and pulmonary embolism (PE) (or, together, venous thromboembolism--VTE). Genomic studies in the general population consistently show that common and low-frequency variants in hemostasis pathway genes are associated with VTE risk.[1] Likewise, epidemiological studies have demonstrated that higher plasma levels of coagulation markers, such as factors VIII and XI, von Willebrand factor, and D-dimer, and shorter activated partial thromboplastin time (aPTT), are associated with increased incidence of VTE.[2,3]

The kallikrein/kinin system, an intravascular biochemical pathway that includes several proteins involved in the contact activation system of coagulation, renin-angiotensin activation, and inflammation,[4] also might play a role in VTE occurrence. High molecular weight kininogen (HK), produced by the gene *KNG1*, serves as a cofactor of factor XIIa in the activation of prekallikrein to kallikrein, an activation that is also stimulated by prolylcarboxypeptidase. Kallikrein cleaves HK to liberate bradykinin, which binds to receptors that modulate vascular permeability and vasodilation. In the contact activation system, HK helps accelerate factor XIIa formation, which activates factor XI in the coagulation cascade. Kallikrein also helps activate plasminogen to plasmin in the fibrinolytic system.

Although the contact system is essential for in vitro surface-initiated coagulation, as exemplified by the aPTT, the degree to which the contact system has a role in initiating physiologic in vivo coagulation or in the etiology of VTE is unclear. Yet, growing experimental evidence suggests that the contact system may make a contribution to thrombosis.[5] Knockout mice deficient in factor XII, prekallikrein, or HK are protected against artificially induced thrombosis.[6–8] Agents inhibiting the contact pathway also can prevent thrombosis in animals in both venous and arterial vascular beds.[9]

Genomic studies have consistently linked a functional *KNG1* missense variant (rs710446) to VTE occurrence[1] and to the aPTT.[10] This suggests that the kallikrein/kinin system may be involved in VTE through contact activation or the kallikrein/kinin system's other effects. Although higher plasma levels of prekallikrein have been associated with greater arterial cardiovascular disease in a few studies,[11,12] to our knowledge, only one retrospective study has examined the association of plasma HK and prekallikrein with VTE. It found that mean HK was higher in VTE patients than in controls, whereas mean prekallikrein was not

different.[13] However, the VTE patients studied were taking vitamin K antagonists, which might have influenced results.

To more fully test the hypothesis that higher plasma levels of HK or prekallikrein are associated with greater VTE incidence in the general population, we conducted a nested case-cohort study within the prospective Atherosclerosis Risk in Communities (ARIC) cohort.

Methods

Study design and sample

We reported the overall ARIC study design, methods, and VTE incidence rates in detail elsewhere.[14,15] In brief, 15,792 men and women aged 45 to 64 years enrolled in the ARIC study in 1987–1989 (visit 1). ARIC performed subsequent examinations in 1990–92 (visit 2), 1993–95 (visit 3), 1996–98, 2011–13, 2016–2017, as well as annual or semi-annual telephone contact. At each visit, risk factors were measured, and plasma was obtained and stored at −80 degrees C. The institutional review committees at each study center approved the methods, and ARIC staff obtained informed participant consent.

For this report of the association of HK and prekallikrein with VTE, we employed a nested case-cohort study within the ARIC cohort.[16] This is a prospective study design in which the baseline biomarker concentrations are measured and compared between all cohort members who develop VTE and a subcohort selected randomly from the entire cohort. ARIC visit 3 (1993–95) served as the baseline, because citrated plasma from the previous two ARIC visits was in short supply. Following the case-cohort method, we included the incident VTE cases that occurred in the full ARIC cohort between the 1993–95 visit and 2015, along with a subcohort selected as a simple random sample from the full ARIC cohort examined at visit 3. We then measured plasma HK and prekallikrein in stored visit 3 samples from the cases and the subcohort sample.

Figure 1 summarizes our case-cohort study design and exclusions. Of the 12,887 ARIC cohort participants examined at visit 3, 12,825 had citrate plasma samples remaining for assay in 2018. Among these, we selected for assay all participants who had developed VTE in ARIC and 4,195 for the random sample subcohort. The laboratory pulled the visit 3 citrate samples and performed the assays, but 32 participants had insufficient plasma to assay either HK or prekallikrein. From those with results, we excluded 148 whose first VTE occurred before visit 3 (because of our interest in incident VTE after visit 3), 13 who were of race groups other than black or white, 443 who had a history of cancer prior to visit 3, 43 who were taking anticoagulants at visit 3, and 110 missing information on visit 3 covariates used in modeling. This left for analysis 612 incident VTE cases and a subcohort sample of 3,530.

VTE occurrence

ARIC staff contacted participants annually or semi-annually by telephone and asked about all hospitalizations in the previous year. Staff then retrieved hospital records for possible VTE events through 2015. To validate VTE events, two physicians reviewed the records using standardized criteria,[15] requiring positive imaging tests for diagnosis of DVT and PE.

The reviewers sub-classified VTEs as unprovoked (no obvious cause) or provoked (associated with cancer, major trauma, surgery, marked immobility). For this report, we restricted DVTs to those in the lower extremity or vena cava, because upper extremity DVTs were relatively few and almost always the result of venous catheters.

Measurement of hemostatic factors at ARIC visit 3

The Laboratory for Clinical Biochemistry Research at the University of Vermont measured several hemostatic factors. In 2014, the laboratory thawed a citrated plasma sample from 1993–95 twice to measure factor XI and D-dimer, which proved to be VTE risk factors.[3,17] The assay for factor XI was a sandwich ELISA using affinity-purified polyclonal antibodies from Affinity Biologicals (Ancaster, Ontario, CAN), with a coefficient of variation for control samples of 9.6%. D-dimer was measured with an immuno-turbidimetric assay (Liatest D-DI; Diagnostica Stago, Parsippany, NJ), with an analytical coefficient of variation of 4–16%. Blind analysis of 73 pairs of ARIC samples split at the time of blood draw and stored until 2014 yielded an intra-class reliability coefficients of 0.81 for factor XI and 0.92 for D-dimer.

In 2018, the laboratory thawed the samples again for measurement of HK (third thaw) and prekallikrein (fourth thaw). Pilot studies demonstrated that prekallikrein was stable across multiple thaws (mean value for one to four thaws in four samples: 18.8, 20.6, 18.4, and 20.1 μg/mL). In contrast, HK declined 16% across four repeated thaws (mean value for one to four thaws in six samples: 29.4, 26.2, 23.5, and 24.8 μg/mL), but we proceeded with analysis on the assumption that the thaw effect would be uniform across samples.

The laboratory used a single reagent lot for prekallikrein and HK measurements in this report. The assay for prekallikrein was an ELISA using monoclonal antibodies from LifeSpan Biosciences (Seattle, WA) with an average coefficient of variation for control samples of 8.9%. The measurement of HK used the Simple Step ELISA® from Abcam (Eugene, OR), which required a high dilution (1:40,000) and produced an average CV for HK of 16.9%. The Abcam kit suggests a “normal range” for HK based on 10 individuals of 42.8–69.5 μg/mL. This is lower than an average normal value based on 10 subjects of 80 μg/mL, reported by Schmaier et al.[18]

As a further check on repeatability, ARIC staff split a number of EDTA specimens at the time of blood draw, processed the split samples separately, and froze them. When the laboratory analyzed 93 paired, similarly thawed samples randomly and blindly in the batches for this study, the reliability (intraclass correlation) coefficients were r=0.56 for HK and r=0.49 for prekallikrein. The Pearson correlation for prekallikrein reliability was r=0.60.

Measurement of other VTE risk factors

Except where noted, our analysis included VTE risk factors documented previously in ARIC and measured at ARIC visit 3. Participants reported race, smoking status, use of hormone replacement therapy (HRT), use of blood pressure medication, and history of cancer. ARIC staff measured sitting blood pressure thrice after a 5-minute rest via a random-zero sphygmomanometer, and we used the mean of the last two measures. Staff measured weight and height. We defined diabetes as a fasting blood glucose of 126 mg/dl or higher, non-

fasting blood glucose of 200 mg/dl or higher, a self-reported physician diagnosis of diabetes, or use of antidiabetic medication in the past 2 weeks. ARIC isolated genomic DNA and measured five key variants important for VTE: *F5* Leiden rs6025, *F2* rs1799963, *ABO* rs8176719 (O vs. non-O groups), *FGG* rs2066865, and *F11* rs2036914, and a weighted genetic risk score was created, as previously reported.[19] ARIC also assessed the *KNG1* missense variant (rs710446), previously associated in genomic consortia with VTE.[1]

At visit 1, ARIC had measured aPTT, factor VIII, von Willebrand factor, and protein C as previously described.[20,21] At visit 2, ARIC estimated glomerular filtration rate (eGFR) from creatinine using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) algorithm.[22] These were unavailable at ARIC visit 3.

Statistical methods

We used SAS (Statistical Analysis System, SAS Institute, Cary, NC) for analysis and accounted for the case-cohort design. We evaluated potential confounding variables by examining means or frequencies of VTE risk factors across quartiles of HK and prekallikrein in the subcohort random sample. We performed proportional hazards regressions to estimate the hazard ratio (HR) of VTE in relation to HK or prekallikrein (quartiles or continuous), employing Barlow's method for variance estimation for the case-cohort design,[23] which takes into account some VTE cases being in the subcohort and some not. Follow-up for VTE began at ARIC visit 3 and went until the date of first VTE occurrence, loss to follow-up, death, or else December 31, 2015. **Model 1** adjusted for age, race, and sex. **Model 2**, our main model, adjusted for age, race, HRT-sex (women-current user, women-nonuser, women-unknown status, men), weight (continuous), height (continuous), diabetes (yes/no), smoking status (current, former, never), systolic BP (continuous), BP medications (yes no), and visit 2 eGFR (continuous). In supplementary analyses, **Model 3** added visit 3 hemostatic factors (factor XI and D-dimer) and a weighted risk score comprising key genetic variants (*F5, F2, ABO, F11, FGG*), and **Model 4** added ARIC visit 1 hemostatic factors (fVIII, VWF, aPTT, protein C). We also generated restricted cubic splines with knots at the 5th, 27.5th, 50th, 72.5th and 95th percentiles to explore the shape of the association of HK and prekallikrein with VTE risk after adjustment for age, race, and sex.

Results

Correlates of HK and prekallikrein

In the subcohort sample of ARIC participants in 1993–95 (visit 3), the mean value for plasma HK was 23.6 μg/mL (SD, 6.4) and for prekallikrein was 15.1 μg/mL (SD, 9.1). The Spearman correlation between the two was r = 0.30.

As shown in Table 1, higher HK was associated most strikingly in the subcohort sample with the following variables: female sex; greater prevalences of diabetes, HRT use, never smoking, and hypertensive medication use; and higher levels of Factor XI (with the Spearman correlation between HK and Factor XI being r = 0.34). Higher plasma prekallikrein demonstrated many of these associations with potential confounding variables

(Table 2), but was also more common in whites than blacks. The Spearman correlation between prekallikrein and Factor XI was r = 0.32. In addition, both higher HK and prekallikrein in 1993–95 were associated with higher levels of Factor VIII and shorter aPTT at ARIC visit 1 in 1987–89.

Greater numbers of risk alleles for *KNG1* rs710446 were associated, as expected, with higher plasma concentrations of HK and prekallikrein in the subcohort sample. Participants with 0, 1, or 2 risk alleles had mean HK concentrations of 22.3, 23.9, and 25.1 μg/mL and mean prekallikrein of 14.1, 15.1, and 16.6 μg/mL, respectively.

Associations of HK and prekallikrein with incident VTE

Over a mean of 18 years of follow-up (maximum 23 years), 612 participants developed VTE, for a crude incidence rate of 3 per 1000 person-years. Weighted for the case-cohort sampling, we found no evidence that plasma HK or prekallikrein was associated positively with incident VTE (Figure 2, Table 3). HK, in fact, was associated inversely and significantly with VTE in most models (Figure 2, Table 3). These conclusions were unchanged by restricting follow-up for VTE to a maximum of 10 years after HK and prekallikrein measurement: the Model 2 hazard ratio for VTE per standard deviation increment of HK being 0.88 (95% CI 0.81–0.97) for full follow-up (Table 3) versus 0.82 (0.69–0.97) for the first 10 years of follow-up (data not shown). Tests of multiplicative interactions of HK or prekallikrein with sex, HRT, factor XI, and D-dimer in relation to VTE were non-significant (p.0.05, data not shown).

Examining the outcomes of DVT or PE separately (Table 4) or provoked versus unprovoked VTE (Table 5), there still was no positive association with either plasma marker. Rather, HK was inversely associated with all VTE outcomes—statistically significantly so for DVT alone and provoked VTE.

Associations of KNG1 rs710446 variant with VTE, with and without adjustment for HK

Given no association of plasma prekallikrein with VTE, it could not mediate the association of *KNG1* rs710446 with VTE.[1] Similarly, given a possible inverse association of plasma HK with VTE, it seemed highly unlikely that HK could mediate the association of *KNG1* rs710446 with VTE[1] either, but we ran a “mediation” model to verify this. In a proportional hazards model, adjusted for age, race and sex but not HK, the HRs (95% CI) of VTE were 1 (reference), 1.12 (0.90, 1.39), and 1.24 (0.96, 1.61) for 0, 1, or 2 risk alleles for *KNG1*, and the HR was 1.11 (0.98, 1.27) per each *KNG1* risk allele treated as an ordinal variable. When HK was added as a continuous variable to the model, these HRs of VTE changed little [1 (reference), 1.16 (0.93, 1.44), and 1.31 (1.00, 1.71) for 0, 1, or 2 *KNG1* risk alleles; 1.15 (1.00, 1.31) per each risk allele]. Thus, plasma HK did not mediate the association of *KNG1* rs710446 with incident VTE.

Discussion

This prospective study showed no significant positive association of plasma levels of HK or prekallikrein with incident VTE in the general population. If anything, HK was inversely associated with VTE. This finding was contrary to our hypothesis of a positive association

derived from several lines of evidence: animal studies of contact system factors and thrombosis,[5–9] genome-wide association studies linking a *KNG1* missense variant (rs710446) with VTE,[1] and a single previous study showing a positive association of HK with VTE.[13] Although we measured just two factors in the kallikrein/kinin system, our findings do not support that higher levels of these two factors in the general population influence the occurrence of VTE. Our findings further suggest that plasma levels of HWMK or prekallikrein do not explain the documented association of *KNG1* rs710446 with VTE.

We have no firm explanation for the graded inverse association between HK and VTE. There is very little prior literature on whether HK and prekallikrein are correlated with other VTE risk factors, which might confound associations with VTE. We, in fact, found HK and prekallikrein associated with several factors (Table 1), but adjustment for them did not greatly change the inverse association of HK and prekallikrein. Nevertheless, there still may have been unidentified confounding, or the inverse association may have been a chance finding, so it certainly warrants replication. In any case, we can speculate about some possible mechanisms. Since HK is a multifunctional protein,[24] an inverse association with VTE could be mediated through pathways other than the contact coagulation system. We observed that D-dimer was lower with increasing HK, supporting the notion that higher HK would be inversely associated with VTE insofar as higher D-dimer is a VTE risk factor. An in vitro study reported HK can inhibit thrombin-induced platelet aggregation by impairing thrombin binding to platelets.[25] Another in vitro study showed that cleaved HK (i.e., the kinin-free form) could interfere with the function of plasminogen activator inhibitor-1,[26] an important regulator of the fibrinolytic system. Experimental rat models of spontaneous kininogen deficiency, due to a point mutation in kininogen, have reported kininogens exert antithrombotic effects in arterial thrombosis.[27,28]

In addition, kininogens are inhibitors of cysteine cathepsins, a group of cysteine proteases.[24] Falanga et al reported that cancer cells expressed a cysteine protease that can directly activate factor X in the absence of factor VII.[29] Cancer is a major contributor to provoked VTE. Other cysteine cathepsins are involved in a variety of physiological function and pathological conditions, including cancer, atherosclerosis and inflammatory diseases,[30] which might contribute to VTE.

Although our study was large, population based, and prospective, it had some drawbacks. Firstly, samples were stored approximately 24 years at −80˚C and previously thawed. As described in Methods, laboratory variability for both HK and prekallikrein was high and pilot studies suggested that HK concentrations in citrate plasma fall somewhat with freeze-thaw cycles. The laboratory imprecision would tend to obscure associations with VTE, but drift from multiple thaws might be expected to have limited impact on associations, as all samples were handled similarly during freeze-thaw cycles. Secondly, the mean value of HK was much lower than a previously established mean value from healthy volunteers of 80 μg/mL.[18] The Abcam assay we chose seems to run low (see Methods), but alternatively ARIC samples may have deteriorated while frozen for approximately 14 years. Assuming the degree of underestimation of concentrations would be comparable in those who did versus did not develop VTE, systematic underestimation of HK should have led to little bias in the hazard ratios.Nevertheless, our findings require replication.

Thirdly, we had only single measures of HK and prekallikrein. During several decades of follow-up, ARIC participants' usual plasma levels of these biomarkers likely changed, but we have no follow-up measures to verify how much. Such within-person variability likely would be random with respect to VTE occurrence, and thus would tend to obscure any real association between the factors and VTE. Fourthly, we identified hospitalized VTE patients only, but pilot data suggest that the vast majority of first VTEs in ARIC were hospitalized.

In conclusion, plasma concentrations of HK and prekallikrein were not positively associated with incidence of VTE, but rather there was an inverse association for HK. Our study suggests that plasma levels of these factors do not determine the risk of VTE in the general population.

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Summary Table

What is known about this topic?

- Whether the kallikrein/kinin and contact activation systems play a role in venous thromboembolism (VTE) is uncertain, although a *KNG1* mutation is associated with VTE.
- A few epidemiological studies have provided mixed evidence that higher plasma concentrations of high molecular weight kininogen (HK) or prekallikrein may increase VTE risk.

What does this paper add?

- Our large prospective study found that higher concentrations of HK and prekallikrein were not associated with greater VTE risk.
- In fact, HK was associated inversely with VTE.
- Our findings suggest that plasma levels of these factors do not determine the risk of VTE in the general population.

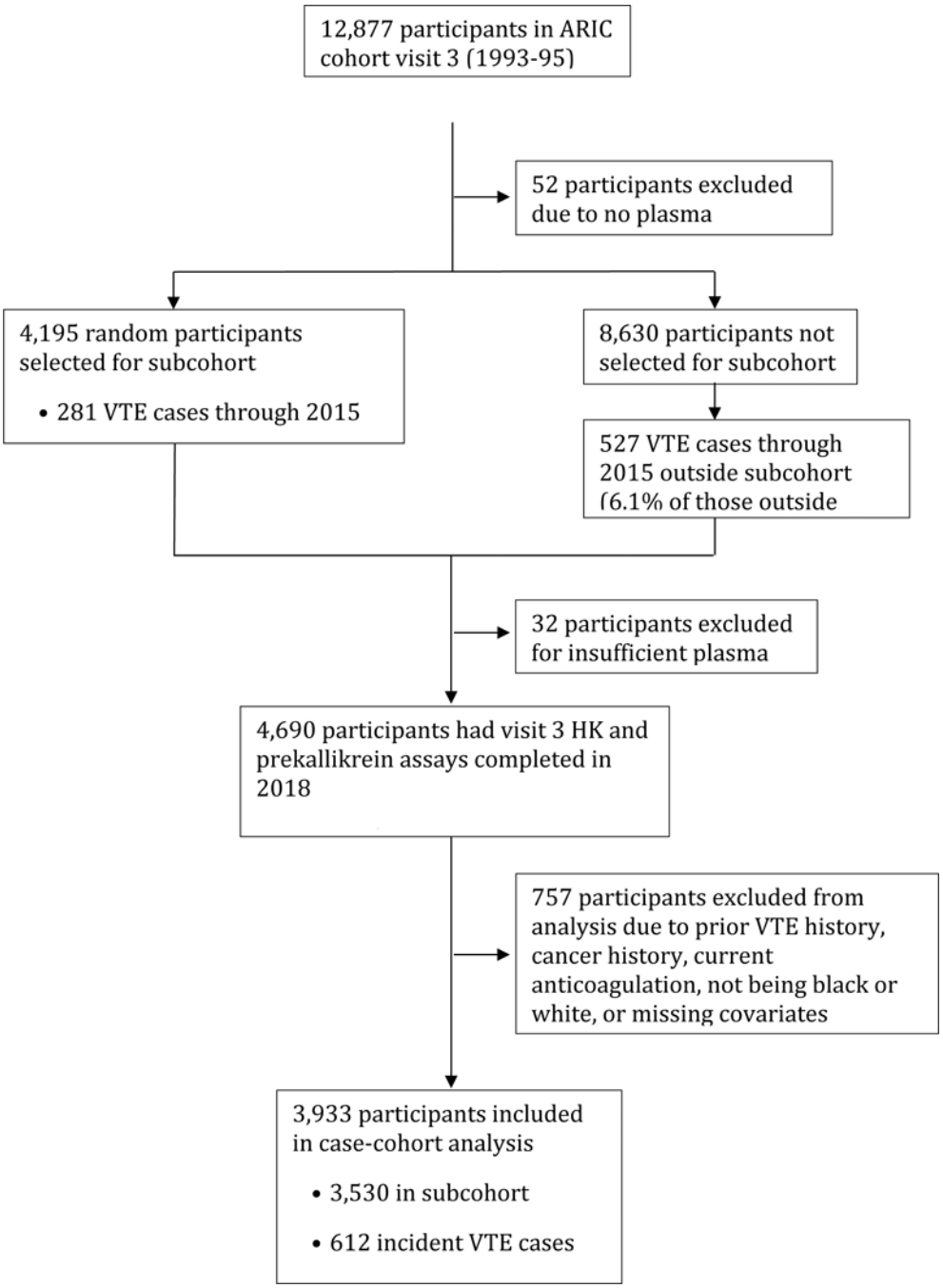

Figure 1.

Nested case-cohort study design and exclusions, ARIC. Abbreviations: ARIC = Atherosclerosis Risk in Communities; HK = high molecular weight kininogen; VTE = venous thromboembolism.

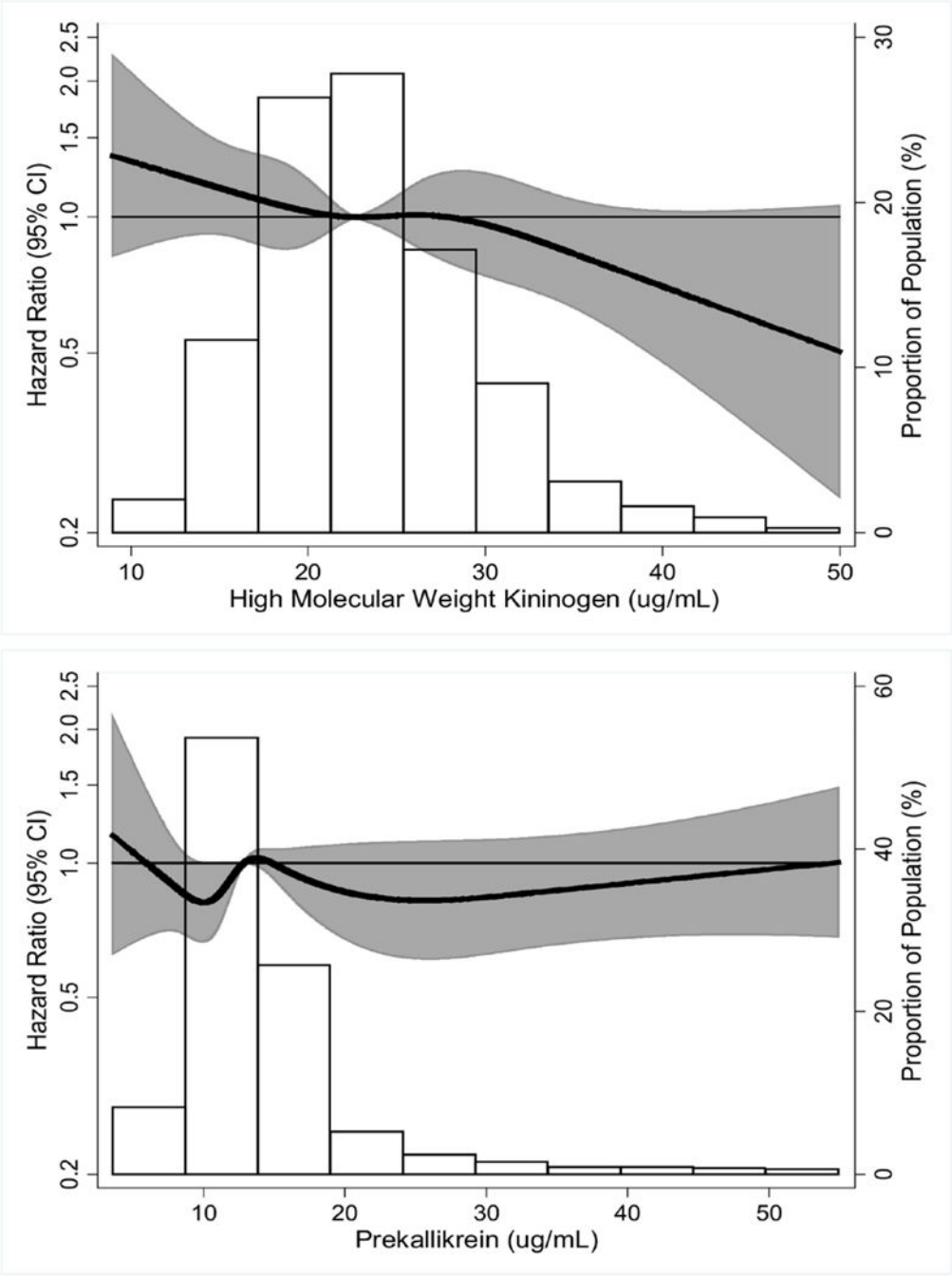

Figure 2.

Hazard ratio of venous thromboembolism in relation to plasma high molecular weight kininogen (top) and prekallikrein (bottom).

Footnote: Hazard ratios (dark line) and 95 percent confidence interval (shaded area) were derived from restricted cubic spline models with knots at the 5th, 27.5th, 50th, 72.5th, and 95th percentiles of the distributions (histograms). For ease of presentation, we omitted

depiction of the curve at extreme values: HK <5 μg/mL (*n*=2) or >50 μg/mL (*n*=12) and prekallikrein <3 μg/mL (*n*=3) or >55 μg/mL (*n*=45).

Table 1

Baseline venous thromboembolism risk factor levels (mean or %) by quartiles of plasma high molecular weight kininogen (HK), ARIC subcohort random sample, 1993−95

	Quartiles			
	Q1	**Q2**	**Q3**	**Q4**
n	871	870	887	902
HK range, μg/mL	2.08−19.11	19.12−22.66	22.67−26.76	26.77−58.57
Age, years	60	60	60	59
Sex, %				
Male	54	47	44	34
Female	46	53	56	66
Race, %				
White	76	78	78	77
African American	24	22	22	23
Height, cm	170	169	168	167
Weight, kg	81	81	80	81
Diabetes, %	12	12	17	23
Hormone replacement therapy, % (females only)	14	17	18	21
Smoking status, %				
Current	20	17	19	16
Former	40	43	41	40
Never	40	40	40	44
Systolic blood pressure, mmHg	124	124	125	123
Antihypertensive medication, %	35	33	37	43
Estimated glomerular filtration rate, mL/min/1.73 m^2 [2]	97	96	97	97
Factor XI, %	102	111	117	125
D-Dimer, μg/mL [1]	0.49	0.43	0.46	0.43
Activated partial thromboplastin time, seconds [1,3]	29.6	29.2	28.9	28.5
Factor VIII, % [1,3]	125	128	128	134
Protein C, μg/mL [1,3]	3.0	3.1	3.2	3.3
Von Willebrand factor, % [1,3]	114	113	114	117
Weighted genetic risk score (*F5, F2, ABO, F11, FGG* variants) [1]	1.38	1.44	1.44	1.52

[1] Sample size modestly smaller due to additional missing data.

[2] From ARIC visit 2, 1990−92.

[3] From ARIC visit 1, 1987−89.

Abbreviations: ARIC = Atherosclerosis Risk in Communities; HK = high molecular weight kininogen

Table 2

Baseline venous thromboembolism risk factor levels (mean or %) by quartiles of plasma prekallikrein, ARIC subcohort random sample, 1993–95

	Quartiles			
	Q1	**Q2**	**Q3**	**Q4**
n	873	898	872	887
Prekallikrein range, μg/mL	2.08−10.59	10.60−12.80	12.81−15.70	15.71−95.79
Age, years	60	60	60	59
Sex, %				
Male	63	56	37	24
Female	37	44	63	76
Race, %				
White	67	78	83	80
African American	33	22	17	20
Height, cm	171	170	167	165
Weight, kg	81	82	80	79
Diabetes, %	13	16	17	18
Hormone replacement therapy, % (females only)	8	10	18	35
Smoking status, %				
Current	24	19	16	12
Former	43	42	39	40
Never	33	38	46	48
Systolic blood pressure, mmHg	125	123	124	125
Antihypertensive medication, %	37	34	37	41
Estimated glomerular filtration rate, mL/min/1.73 m^2 [2]	98	96	97	97
Factor XI, %	103	110	118	124
D-Dimer, μg/mL [1]	0.49	0.42	0.37	0.54
Activated partial thromboplastin time, seconds [1,3]	29.9	29.2	28.9	28.1
Factor VIII, % [1,3]	129	126	129	132
Protein C, μg/mL [1,3]	3.0	3.1	3.2	3.3
Von Willebrand factor, % [1,3]	119	113	113	113
Weighted genetic risk score (*F5, F2, ABO, F11, FGG* variants) [1]	1.43	1.42	1.46	1.48

[1] Sample size modestly smaller due to additional missing data.

[2] From ARIC visit 2, 1990−92.

[3] From ARIC visit 1, 1987−89.

Abbreviations: ARIC = Atherosclerosis Risk in Communities

Table 3

Hazard ratios (95% confidence intervals) of venous thromboembolism (VTE) by quartiles or SD increment of high molecular weight kininogen and prekallikrein, ARIC, 1993–95 through 2015

	Quartiles				
	Q1	**Q2**	**Q3**	**Q4**	**Per SD increment**[2]
High molecular weight kininogen, μg/mL	2.08−19.11	19.12−22.66	22.67−26.76	26.77−58.57	
n at risk	983	983	984	983	3,933
Incident VTE, n	172	161	141	138	612
Model 1 HR (95% CI)	1 (Reference)	0.91 (0.72−1.15)	0.85 (0.67−1.08)	0.79 (0.62−1.01)	0.89 (0.82−0.97)
Model 2 HR (95% CI)	1 (Reference)	0.90 (0.71−1.14)	0.84 (0.66−1.07)	0.77 (0.60−0.99)	0.88 (0.81−0.97)
Model 3 HR (95% CI)[1]	1 (Reference)	0.93 (0.72−1.21)	0.79 (0.60−1.04)	0.68 (0.51−0.90)	0.83 (0.75−0.92)
Model 4 HR (95% CI)[1]	1 (Reference)	0.93 (0.71−1.21)	0.80 (0.61−1.06)	0.67 (0.50−0.90)	0.83 (0.75−0.92)
Prekallikrein, μg/mL	2.08−10.59	10.60−12.80	12.81−15.70	15.71−95.79	
n at risk+	983	983	985	982	3,933
Incident VTE, n	156	147	163	146	612
Model 1 HR (95% CI)	1 (Reference)	0.96 (0.75−1.22)	1.17 (0.92−1.49)	0.99 (0.77−1.27)	1.00 (0.91−1.09)
Model 2 HR (95% CI)	1 (Reference)	0.97 (0.76−1.24)	1.14 (0.89−1.46)	0.95 (0.73−1.23)	0.99 (0.91−1.09)
Model 3 HR (95% CI)[1]	1 (Reference)	1.03 (0.78−1.36)	1.20 (0.91−1.59)	1.05 (0.79−1.41)	0.95 (0.86−1.06)
Model 4 HR (95% CI)[1]	1 (Reference)	1.03 (0.78−1.36)	1.18 (0.89−1.57)	1.04 (0.78−1.40)	0.95 (0.85−1.05)

Model 1: Adjusted for age, race, and sex

Model 2: Adjusted for age, race, sex-hormone replacement therapy, weight, height, diabetes, smoking status, systolic blood pressure, antihypertensive medications, and estimated glomerular filtration rate

Model 3: Model 2 + adjustment for factor XI, log(D-dimer), and weighted genetic risk score (F5, F2, ABO, F11, FGG variants).

Model 4: Model 3 + adjustment for factor VIII, activated partial thromboplastin time, protein C, and Von Willebrand factor.

[1]Models 3 and 4 have a reduced sample size of 3,352 at risk and 514 VTE events due to missing values.

[2]Standard deviation increments were 6.4 μg/mL for HK and 9.1 μg/mL for prekallikrein.

Abbreviations: ARIC = Atherosclerosis Risk in Communities; CI = confidence interval; HK = high molecular weight kininogen; HR = hazard ratio; SD= standard deviation; VTE = venous thromboembolism

Table 4

Hazard ratios (95% confidence intervals) of deep vein thrombosis (DVT) alone and pulmonary embolism (PE, with or without DVT) by quartiles or SD increment of high molecular weight kininogen and prekallikrein, ARIC, 1993–95 through 2015

	Quartiles				
	Q1	**Q2**	**Q3**	**Q4**	**Per SD increment**[2]
High molecular weight kininogen, μg/mL	2.08–19.11	19.12–22.66	22.67–26.76	26.77–58.57	
n at risk[1]	983	983	984	983	3,933
Incident DVT, n	89	80	56	60	285
Model 2 HR (95% CI)	1 (Reference)	0.87 (0.63–1.20)	0.66 (0.46–0.93)	0.67 (0.47–0.94)	0.81 (0.71–0.93)
Model 3 HR (95% CI)	1 (Reference)	0.89 (0.63–1.27)	0.61 (0.41–0.92)	0.58 (0.39–0.87)	0.76 (0.65–0.89)
Incident PE, n	83	81	85	78	327
Model 2 HR (95% CI)	1 (Reference)	0.92 (0.67–1.27)	1.03 (0.75–1.41)	0.88 (0.63–1.22)	0.95 (0.85–1.06)
Model 3 HR (95% CI)	1 (Reference)	0.96 (0.68–1.37)	0.97 (0.68–1.39)	0.78 (0.53–1.14)	0.89 (0.79–1.01)
Prekallikrein, μg/mL	2.08–10.59	10.60–12.80	12.81–15.70	15.71–95.79	
n at risk[1]	983	983	985	982	3,933
Incident DVT, n	84	67	76	58	285
Model 2 HR (95% CI)	1 (Reference)	0.86 (0.62–1.20)	1.10 (0.78–1.54)	0.81 (0.56–1.17)	0.92 (0.78–1.08)
Model 3 HR (95% CI)	1 (Reference)	0.97 (0.66–1.40)	1.24 (0.84–1.81)	0.89 (0.59–1.33)	0.92 (0.78–1.09)
Incident PE, n	72	80	87	88	327
Model 2 HR (95% CI)	1 (Reference)	1.10 (0.78–1.54)	1.19 (0.86–1.66)	1.10 (0.78–1.54)	1.04 (0.93–1.16)
Model 3 HR (95% CI)	1 (Reference)	1.11 (0.76–1.62)	1.18 (0.80–1.73)	1.19 (0.81–1.76)	0.97 (0.86–1.09)

Model 2: Adjusted for age, race, sex-hormone replacement therapy, weight, height, diabetes, smoking status, systolic blood pressure, antihypertensive medications, and estimated glomerular filtration rate

Model 3: Model 2 + adjustment for factor XI, log(D-dimer), and weighted genetic risk score (F5, F2, ABO, F11, FGG variants).

[1]Model 3 has a reduced sample size due to missing values (see Table 3). Model 1 findings were similar to Model 2 and Model 4 findings were similar to Model 3 and thus not shown.

[2] Standard deviation increments were 6.4 μg/mL for HK and 9.1 μg/mL for prekallikrein.

Abbreviations: ARIC = Atherosclerosis Risk in Communities; CI = confidence interval; DVT = deep vein thrombosis; HK = high molecular weight kininogen; HR = hazard ratio; PE = pulmonary embolism; SD = standard deviation.

Table 5

Hazard ratios (95% confidence intervals) of provoked and unprovoked venous thromboembolism (VTE) by quartiles or SD increments of high molecular weight kininogen and prekallikrein, ARIC, 1993–95 through 2015

	Quartiles				
	Q1	**Q2**	**Q3**	**Q4**	**Per SD increment**[2]
High molecular weight kininogen, μg/mL	2.08–19.11	19.12–22.66	22.67–26.76	26.77–58.57	
n at risk[1]	983	983	984	983	3,933
Incident Provoked VTE, n	102	104	84	77	367
Model 2 HR (95% CI)	1 (Reference)	0.97 (0.73–1.30)	0.84 (0.62–1.14)	0.71 (0.52–0.98)	0.86 (0.77–0.96)
Model 3 HR (95% CI)	1 (Reference)	1.00 (0.73–1.37)	0.78 (0.55–1.11)	0.64 (0.44–0.93)	0.82 (0.73–0.94)
Incident Unprovoked VTE, n	70	57	57	61	245
Model 2 HR (95% CI)	1 (Reference)	0.79 (0.54–1.13)	0.85 (0.59–1.23)	0.86 (0.60–1.24)	0.93 (0.81–1.06)
Model 3 HR (95% CI)	1 (Reference)	0.82 (0.54–1.23)	0.80 (0.52–1.21)	0.74 (0.49–1.12)	0.85 (0.73–0.99)
Prekallikrein, μg/mL	2.08–10.59	10.60–12.80	12.81–15.70	15.71–95.79	
n at risk[1]	983	983	985	982	3,933
Incident Provoked VTE, n	103	87	96	81	367
Model 2 HR (95% CI)	1 (Reference)	0.87 (0.65–1.18)	1.03 (0.76–1.41)	0.80 (0.58–1.11)	0.96 (0.84–1.09)
Model 3 HR (95% CI)	1 (Reference)	0.90 (0.64–1.25)	1.11 (0.79–1.57)	0.86 (0.59–1.25)	0.92 (0.80–1.05)
Incident Unprovoked VTE, n	53	60	67	65	245
Model 2 HR (95% CI)	1 (Reference)	1.16 (0.79–1.70)	1.34 (0.92–1.95)	1.24 (0.84–1.82)	1.04 (0.91–1.18)
Model 3 HR (95% CI)	1 (Reference)	1.34 (0.86–2.11)	1.40 (0.90–2.18)	1.47 (0.96–2.26)	1.00 (0.86–1.15)

Model 2: Adjusted for age, race, sex-hormone replacement therapy, weight, height, diabetes, smoking status, systolic blood pressure, antihypertensive medications, and estimated glomerular filtration rate

Model 3: Model 2 + adjustment for factor XI, log(D-dimer), and weighted genetic risk score (F5, F2, ABO, F11, FGG variants).

[1]Model 3 has a reduced sample size due to missing values (see Table 3). Model 1 findings were similar to Model 2 and Model 4 findings were similar to Model 3 and thus not shown.

[2] Standard deviation increments were 6.4 μg/mL for HK and 9.1 μg/mL for prekallikrein.

Abbreviations: ARIC = Atherosclerosis Risk in Communities; CI = confidence interval; HK = high molecular weight kininogen; HR = hazard ratio; SD = standard deviation; VTE = venous thromboembolism