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ORIGINAL ARTICLE

Associations of coagulation factor X and XI with incident acute coronary syndrome and stroke: A nested case-control study

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Abstract

Background: Coagulation cascade contributes to thrombotic and hemorrhagic diseases, but it remains unclear whether coagulation factors X (FX) and XI (FXI) levels are associated with cardiovascular diseases.

Objective: To evaluate prospective associations of FX and FXI levels with incident acute coronary syndrome (ACS), stroke, and their subtypes (acute myocardial infarction, unstable angina, ischemic stroke, and hemorrhagic stroke).

Methods: We performed a nested case-control study ($n = 1846$) within the Dongfeng-Tongji cohort from 2013 to 2016 matched on age (within 1 year), sex, and sampling date (within 1 month) by incidence density sampling, and measured plasma FX and FXI levels by enzyme-linked immunosorbent assay. FX and FXI levels were categorized into three groups (low, <25th; middle, 25th to <75th; and high ≥75th percentiles) according to distributions, and conditional logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs).

Results: After adjustment for traditional cardiovascular risk factors, compared with middle groups, the OR (95% CI) in high levels of FX and FXI were 1.11 (0.79–1.56) and 0.96 (0.68–1.36) for incident ACS, and 1.01 (0.63–1.62) and 1.72 (1.14–2.60) for incident stroke, respectively. As for subtypes of ACS and stroke, only high FXI levels were significantly associated with incident ischemic stroke (OR 1.66, 95% CI 1.05–2.65). Moreover, all associations remained steady after additional adjustment for platelet and leukocyte.

Conclusion: FXI levels were associated with a greater risk of incident ischemic stroke but not hemorrhagic stroke or ACS. FX levels were not associated with incident ACS or stroke.

KEYWORDS
acute coronary syndrome, coagulation factor X, coagulation factor XI, prospective study, stroke

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1 | INTRODUCTION

Coronary heart disease (CHD) and stroke are the major contributors to the global burden of disease[1] and the leading causes of death and disability-adjusted life-years in China.[2] Acute coronary syndrome (ACS) is a subtype of CHD that has a high mortality rate. Thrombosis and fibrinolysis disorder may be involved in the pathophysiological process of ACS and stroke.[3,4] Existing studies have shown that anticoagulation factor medication might be a therapy for recurrent thrombotic events,[5,6] and some coagulation factors were significantly associated with venous thrombosis.[7,8] However, prospective studies focused on the associations of coagulation factor X (FX) and XI (FXI) with ACS, stroke, and their subtypes (acute myocardial infarction, unstable angina, ischemic stroke, and hemorrhagic stroke) were limited.

FX occupied a central position as a convergence point between the intrinsic and extrinsic coagulation pathways and played a pivotal role in thrombosis and fibrinolysis disorder.[9] To our knowledge, only two case cohort studies based on community reported the prospective association of FX levels with incident CHD or ischemic stroke, but no significant result was observed.[10,11] Moreover, although FX activity levels were related to bleeding severity in rare bleeding disorders,[12] and targeting FX medication (e.g., warfarin) therapy was often accompanied by bleeding side effects[5]; thus, it the relationship between FX and hemorrhagic stroke was unknown.

FXI participated in the early phase of thrombosis and was taken to be a novel target for thrombus therapy.[13] Previous prospective studies regarding the associations between FXI levels and incident CHD or stroke were inconsistent.[10,11,14-16] A nested case-control study and a study of prognosis observed that FXI was associated with higher risk of incident ischemic stroke or secondary events after ischemic stroke.[10,14] On the contrary, three studies based on cohort reported that the association of basal FXI levels with CHD or ischemic stroke was not significant.[11,15,16] The disparate results might be due to different study design, population characteristics, and storage duration for samples. Because of the low incidence rate and high mortality rate, only two prospective studies reported the association between FXI and hemorrhagic stroke but without significance.[15,17]

However, these limited prospective evidences were mostly from European and American populations, and few studies focused on subtypes of ACS and stroke. Therefore, we performed the nested case-control study within a prospective cohort to systematically explore the prospective associations between FX, FXI levels and risks of incident ACS, stroke, and their subtypes among Chinese.

Essentials

- It remains unclear whether coagulation factors X (FX) and XI (FXI) levels are associated with cardiovascular diseases.
- The association of FXI with incident stroke might be attributed to ischemic but not hemorrhagic stroke and was robust after additional adjustment for platelet, leukocyte, and FX.
- FXI was not associated with incident acute coronary syndrome (ACS) and FX was not associated with incident ACS or stroke.
- The risk of incident stroke with high FXI levels might appear to be more pronounced in participants who were elderly, female, hypertension, hyperlipidemia, and without diabetes mellitus.

2 | METHODS

2.1 | Study design and population

The nested case-control study was based on an ongoing prospective cohort, which has been reported in detail.[18] In brief, the Dongfeng-Tongji cohort recruited 27 009 retirees of Dongfeng Motor Corporation at baseline from September 2008 to June 2010 in Shiyan, and completed the first follow-up from April 2013 to October 2013, which had 14 120 individuals newly recruited. A total of 38 295 participants finished questionnaires and physical examinations in 2013 and were followed until December 31, 2016. We excluded participants who had been diagnosed with cancer, cardiovascular diseases (CVD), or abnormal electrocardiogram (n = 10 254) in the first follow-up (2013). Moreover, we further excluded participants who had a history of medication (thrombolytic, anticoagulant) in the 2 weeks before the survey (n = 752), which might influence coagulation factor levels. Besides, participants who without enough plasma samples for coagulation factors measurement (n = 3812) were also excluded in the study. Finally, a total of 23 477 participants were eligible, of which 549 participants were first diagnosed with incident ACS cases (117 acute myocardial infarction and 432 unstable angina) and 374 with incident stroke cases (295 ischemic stroke and 79 hemorrhagic stroke) at subsequent follow-up from January 10, 2013, to December 31, 2016. Equal number controls were randomly selected from the eligible participants by incidence density sampling, and each case-control pair was matched on age (within 1 year), sex, and sampling date (within 1 month), which minimized confounding effects of matched variables.[19] Therefore, 549 ACS case-control pairs and 374 stroke case-control pairs were included in the nested case-control study. A detailed flowchart of the study participants is provided in Figure S1.

Moreover, the participants of the nested case-control study were selected from the first follow-up, not at baseline, because the samples from baseline were not eligible compared with fresh samples (Table S2). Results were derived from an experiment with small samples (n = 50) comparing with storage for 10 years (baseline), 5 years (first follow-up), and fresh plasma samples.

All participants gave informed consent and these studies were approved by the Ethics and Human Subject Committee of Huazhong University of Science and Technology.

2.2 | Plasma FX and FXI measurements

All fasting plasma samples were separated from EDTA anticoagulation whole blood after centrifuged in the first follow-up (2013) and stored at −80°C until 2019. Plasma samples of case-control pairs were random and laboratory personnel was blinded to disease status. We carried out experiments according to the instruction of the enzyme-linked immunosorbent assay kits, and measured FX and FXI levels on the multifunctional enzyme marker (BioTek) at 450 nm. Samples of quality control were to estimate the stability of experimental operation in all enzyme-linked immunosorbent assay kits (Assaypro). Each sample and its duplicate were measured at the same time.

2.3 | Ascertainment of incident ACS, stroke, and their subtypes

All employees were covered by the Dongfeng Motor Corporation's health care service system and each person had a unique medical insurance card number; therefore, it was easy to track disease incidence. The incidence of ACS and stroke events was identified through this medical insurance system and medical record reviews. ACS was confirmed based on clinical history, symptoms, electrocardiograph, cardiac troponin concentration, coronary angiography, and risk factors according to World Health Organization guidelines, and divided into acute myocardial infarction and unstable angina by tracking the International Classification of Diseases codes-10 (I20-I25).[20,21] Stroke was defined as a sudden or rapid occurrence of a vascular origin nerve defect that lasted for more than 24 h or until death[22] and was diagnosed by the doctor according to the patient's clinical symptoms and imaging examination. Stroke subtypes were further classified by physicians into ischemic stroke and hemorrhagic stroke according to computed tomography, magnetic resonance imaging, and the International Classification of Diseases codes-10 (I60-I61, I63-I64, I69.0-I69.1, and I69.3-I69.4).

2.4 | Assessment of covariates

Trained interviewers collected information of participants through face-to-face interviews by a semistructured questionnaire, including sociodemographic characteristic, lifestyles, family history of disease, and history of medication in the past 2 weeks. Covariates including age, sex, education (primary school or below, middle school, high school, or beyond), physical activity (yes, no), smoking status (current, former, and never), drinking status (current, former, and never), family history of CHD/stroke (yes, no), and use of aspirin (yes, no) were self-reported. Trained professional calculated body mass index (BMI) based on the formula of body weight (kg)/standing height (m^2). The type and duration of physical activity were self-reported and were calculated to metabolic equivalent task hours per week (MET-hours/week) according to formula.[23] Family history of CVD included family history of CHD and stroke.

Physical examinations (e.g., electrocardiogram, computed tomography, magnetic resonance imaging) and all blood biochemical indexes (e.g., blood glucose indexes, lipid indexes, platelet indexes) were detected by trained staff in Sinopharm Dongfeng General Hospital, Shiyan. Participants who with systolic blood pressure ≥140 mmHg, or diastolic blood pressure ≥90 mmHg, or self-reported hypertension, or use of antihypertensive drugs were all defined as hypertension. Participants who with fasting blood glucose ≥7.00 mmol/L, or glycosylated hemoglobin type A1c ≥ 6.5%, or self-reported diabetes mellitus, or taking oral hypoglycemic medication or insulin were defined as diabetes mellitus. Participants who with total cholesterol ≥6.22 mmol/L, or triglyceride ≥2.26 mmol/L, or high-density lipoprotein cholesterol <1.04 mmol/L, or low-density lipoprotein cholesterol ≥4.14 mmol/L, or self-reported hyperlipidemia, or taking lipid-lowering drugs were defined as hyperlipidemia. More details on assessment of covariates were shown in the Supplementary Material.

2.5 | Statistical analysis

Baseline characteristics of the study population were presented as mean with standard deviation or median with interquartile range for continuous variables, and number with percentage for categorical variables. We used the mean or median values as imputation values for missing values of continuous covariates, and an additional category to indicate the missing for missing values of categorical variable. Variables with imputation were just used as covariates in adjustment models.

The restricted cubic splines were to evaluate the dose-response relationships of coagulation factors with incident ACS and stroke according to the distribution of coagulation factors levels using three knots (5th, 50th, and 95th percentiles) and the odds ratio value (OR = 1) at the 10th percentile was considered as reference.[24] Participants were categorized into three groups (low, <25th; middle, 25 to <75th; and high ≥75th percentiles). Besides, these groups were categorized according to the distribution of controls and the curves of restricted cubic spline, and the middle groups were taken as references. For ACS and its subtypes, the middle groups of FX and FXI levels were defined as 7.71 to <10.51 mg/L and 4.36 to <5.92 mg/L, respectively. Likewise, the middle groups of FX and FXI levels were 6.51 to <9.28 mg/L and 3.96 to <5.63 mg/L for stroke and its subtypes.

Conditional logistic regression was to estimate adjusted ORs and 95% confidence intervals (CIs) between FX and FXI levels and ACS, stroke, and their subtypes by multiadjustment. Covariates including age, sex, BMI, smoking status, drinking status, physical activity (MET-hours/week), hypertension, diabetes mellitus, hyperlipidemia, and use of aspirin were adjusted in model 1. Platelet and leukocyte might be involved in the thrombosis, and were associated with higher risk of CVD.[25-27] To clarify whether the associations of coagulation factors with incident ACS and stroke were influenced by these factors, model 2 was additionally adjusted for platelet and leukocyte

(FX and FXI were adjusted for each other in model 2). Moreover, we also plotted Kaplan-Meier curves based on the follow-up (years) to estimate the cumulative percent of incident ACS, stroke, and their subtypes between groups of FX and FXI levels by log-rank test without adjustment.

Previous studies indicated that coagulation factors combined with certain characteristic (e.g., hyperlipidemia, headache) had particularly higher effects on CVD.[28,29] We further performed stratified analyses according to traditional CVD risk factors including age (<65, ≥65 years), sex (male, female), hypertension (yes, no), hyperlipidemia (yes, no), diabetes mellitus (yes, no), smoking status (yes, no), and drinking status (yes, no) to explore possible effect modifications using unconditional logistic regression. Potential interaction effects were estimated by adding multiplicative interaction terms of these covariates. In the stratified analysis, smoking status (yes) was combined with current and former smokers. Similarly, drinking status (yes) was combined with current and former drinkers. The person-year for each participant was calculated from the date of the first follow-up to the date of diagnosis of ACS or stroke, or the end of December 31, 2016.

Sensitivity analyses were performed to estimate the robustness of results. First, we excluded the case-control pairs with missing data of covariates to further clarify the impact of missing data on the results. Moreover, we excluded case-control pairs within half a year of follow-up to clarify whether these associations resulted from the acute phase. Besides, we excluded case-control pairs missing liver enzymes, and additionally adjusted for liver dysfunction (yes, no) in the sensitivity analysis because the liver might be an organ that produced coagulation factors.[30]

All analyses were performed using SAS software (version 9.4, SAS Institute Inc. Cary, NC) and R software (version 3.5.0; R Core Team). A two-sided $p < 0.05$ was considered as statistical significant.

3 | RESULTS

3.1 | Baseline characteristics

The nested case-control study included 1846 participants (549 incident ACS cases, 374 incident stroke cases, and 923 matched controls) with 59% male and mean age 67 years during a median follow-up of 2.7 years. The baseline characteristics of participants are shown in Table 1. Compared with matched controls, we observed that elevated BMI, higher proportions of smokers, hypertension, hyperlipidemia, diabetes mellitus, and aspirin users in the incident ACS cases. Meanwhile, the incident ACS cases were more likely to have higher FX levels and more leukocytes. Likewise, the incident stroke cases were more likely to have higher BMI, FX levels, FXI levels, and more leukocytes than matched controls. Moreover, the proportions of hypertension, hyperlipidemia, diabetes mellitus, and family history of CVD were higher in incident stroke cases. The subtypes of ACS (acute myocardial infarction and unstable angina) and stroke (ischemic stroke and hemorrhagic stroke) show similar baseline characteristics in Table S1.

3.2 | Associations of FX and FXI levels with ACS, stroke, and their subtypes

The restricted cubic splines also showed that linear relationships of FX and FXI levels with incident ACS and stroke were not statistically significant (all $p > 0.05$; Figures S2 and S3), which might suggest nonline relationships with “J” or "U" shape curves. As shown in Table 2, compared with participants with middle FX levels, the multivariate-adjusted ORs (95% CIs) of incident ACS were 1.27 (0.88–1.84) for participants with low levels and 1.11 (0.79–1.56) for high levels. For incident stroke, adjusted ORs (95% CIs) were 1.07 (0.71–1.64) for participants with low FX levels and 1.01 (0.63–1.62) for high FX levels. Moreover, we did not observe any significant association of FX levels with subtypes of ACS or stroke. The associations for FX levels with ACS, stroke, and their subtypes in model 2 were all not significant, which was similar to the primary results in model 1 (Table 2). Compared with the middle groups of FXI levels, the multivariate-adjusted ORs (95% CIs) of incident ACS were 1.14 (0.81–1.60) for low group and 0.96 (0.68–1.36) for high group. With respect to incident stroke, the adjusted ORs (95% CIs) were 1.16 (0.75–1.79) and 1.72 (1.14–2.60) for low and high groups of FXI after adjusted traditional cardiovascular risk factors in conditional logistic regression. When stratified by subtypes of ACS and stroke, a similar association was observed between high FXI levels and incident ischemic stroke (OR 1.66, 95% CI 1.05–2.65, $p = 0.032$), but not hemorrhagic stroke (OR 1.95, 95% CI 0.65–5.82), acute myocardial infarction (OR 0.69, 95% CI 0.32–1.56), or unstable angina (OR 1.02, 95% CI 0.69–1.52). After additional adjustment for platelet, leukocyte, and FX, the associations of high FXI levels with incident stroke and ischemic stroke were slightly attenuated (stroke: OR 1.69, 95% CI 1.11–2.58; ischemic stroke: OR 1.63, 95% CI 1.02–2.62).

From the Kaplan-Meier curves, a significant difference was observed between groups of FXI for cumulative percent of incident stroke ($p = 0.030$) during the follow-up duration, whereas groups of FX had no significant difference for cumulative percent of incident stroke, as well as ACS (Figure 1). With respect to subtypes of ACS and stroke, marginal difference for cumulative percent of incident ischemic stroke ($p = 0.075$) between groups of FXI levels during the follow-up without adjustment (Figure S4). Furthermore, the Kaplan-Meier curves plots also shown that high group of FXI levels had the highest cumulative percent of incident stroke and ischemic stroke compared with low and middle groups during the follow-up years (Figure 1 and S4).

Stratified analyses of demographic characteristics, basic disease, and lifestyles are shown in Figure 2. The associations between high FXI levels and incident stroke were more pronounced in subgroups of age more than 65 years old, female, hypertension, hyperlipidemia, and without diabetes mellitus (all $p < 0.05$), but the interaction effects were not significant ($p > 0.05$). The associations between high

TABLE 1 Baseline characteristics of ACS and stroke in the nested case-control study.

	ACS		Stroke	
	Cases ($n = 549$)	Controls ($n = 549$)	Cases ($n = 374$)	Controls ($n = 374$)
Age (years)	66.94 ± 8.01	66.92 ± 8.04	67.74 ± 7.66	67.70 ± 7.63
Male, *n* (%)	301 (54.8)	301 (54.8)	246 (65.8)	246 (65.8)
BMI (kg/m^2)[a]	24.60 ± 3.12	24.03 ± 3.19	24.64 ± 3.31	24.17 ± 3.12
Smoking status, *n* (%)				
Current smoker	125 (22.8)	97 (17.7)	110 (29.4)	88 (23.5)
Former smoker	88 (16.0)	77 (14.0)	59 (15.8)	55 (14.7)
Never	336 (61.2)	375 (68.3)	205 (54.8)	231 (61.8)
Drinking status, *n* (%)[a]				
Current drinker	153 (27.9)	150 (27.3)	114 (30.5)	108 (28.9)
Former drinker	38 (6.9)	27 (4.9)	29 (7.8)	28 (7.5)
Never	357 (65.0)	372 (67.8)	231 (61.8)	238 (63.6)
Education level, *n* (%)[a]				
Primary school or below	165 (30.1)	143 (26.0)	105 (28.1)	105 (28.1)
Middle school	218 (39.7)	215 (39.2)	146 (39.0)	130 (34.8)
High school or beyond	165 (30.1)	191 (34.8)	121 (32.4)	139 (37.2)
Hypertension, *n* (%)	423 (77.0)	334 (60.8)	302 (81.7)	237 (63.4)
Hyperlipidemia, *n* (%)	291 (53.0)	230 (41.9)	191 (51.1)	145 (38.8)
Diabetes mellitus, *n* (%)	158 (28.8)	116 (21.1)	127 (34.0)	83 (22.2)
Family history of CVD, *n* (%)	52 (9.5)	55 (10.0)	25 (6.7)	40 (10.7)
Physical activity, *n* (%)	478 (87.1)	490 (89.3)	329 (88.0)	331 (88.5)
Physical activity, (MET-hours/week)	21 (11.6–42.0)	21 (12.0–42.0)	21 (9.0–42.0)	23.8 (10.5–42.0)
Use of aspirin	78 (14.2)	43 (7.8)	43 (11.5)	33 (8.8)
Platelet (10^9/L)	191 (160–225)	191 (160–221)	193 (162–224)	186 (159–221)
Leukocyte (10^9/L)	5.93 ± 1.56	5.72 ± 1.49	6.05 ± 1.60	5.80 ± 1.48
FX levels (mg/L)	9.52 ± 2.59	9.22 ± 2.33	8.44 ± 2.34	8.09 ± 2.36
FXI levels (mg/L)	5.22 ± 1.26	5.18 ± 1.26	5.13 ± 1.45	4.90 ± 1.32

Note: Variables were shown as percentage for categorical variables and mean ± standard deviation or median (interquartile range, 25th-75th percentiles) for continuous variables.

Abbreviations: ACS, acute coronary syndrome; BMI; body mass index; FX, coagulation factor X; FXI, coagulation factor XI; METS, metabolic equivalent task hours.

[a]Data were incomplete for these variables. A total of 35 (1.89%), 1 (0.05%), and 3 (0.16%) of participants had missing data for BMI, drinking status, and education levels in the nested case-control study.

FXI levels and incident ACS were not significant in all stratified subgroups, which were similar to FX levels (Figure 2 and S5).

In sensitivity analyses, the associations of FXI levels with stroke and ischemic stroke were not substantially changed when excluded case-control pairs with missing covariates or within half a year of follow-up (Table S3 and S4). Similarly, additional adjustment for liver dysfunction after excluding case-control pairs missing liver enzymes did not alter the risk of FXI levels with incident stroke or ischemic stroke substantially (Table S5).

4 | DISCUSSION

In the nested case-control study within the Dongfeng-Tongji cohort, we examined the associations of FX and FXI levels with risk of ACS, stroke, and their subtypes. We found that the association of FXI with incident stroke might be attributed to ischemic stroke but not hemorrhagic stroke and was robust to further adjust for platelet, leukocyte, and FX. In contrast, FXI was not associated with incident ACS and FX was not associated with incident ACS or stroke. Moreover,

TABLE 2 Adjusted odds ratios (ORs) for incident ACS, stroke, and their subtypes according to coagulation factors in the nested case-control study

	FX levels, mg/L			FXI levels, mg/L		
	Low	Middle	High	Low	Middle	High
	<7.71	7.71 to <10.51	≥10.51	<4.36	4.36 to <5.92	≥5.92
ACS						
Cases/controls	136/136	255/275	158/138	128/133	281/279	140/137
Model 1	1.27 (0.88–1.84)	Reference	1.11 (0.79–1.56)	1.14 (0.81–1.60)	Reference	0.96 (0.68–1.36)
Model 2[a]	1.26 (0.86–1.85)	Reference	1.12 (0.78–1.60)	1.22 (0.86–1.74)	Reference	0.91 (0.63–1.30)
AMI						
Cases/controls	27/27	53/58	37/32	33/30	58/58	26/29
Model 1	1.31 (0.52–3.23)	Reference	1.26 (0.56–2.86)	1.25 (0.57–2.77)	Reference	0.69 (0.32–1.56)
Model 2[a]	1.28 (0.49–3.30)	Reference	1.32 (0.55–3.18)	1.74 (0.73–4.16)	Reference	0.57 (0.24–1.36)
UA						
Cases/controls	109/109	202/217	121/106	95/103	223/221	114/108
Model 1	1.22 (0.80–1.86)	Reference	1.11 (0.75–1.64)	1.05 (0.71–1.56)	Reference	1.02 (0.69–1.52)
Model 2[a]	1.22 (0.79–1.89)	Reference	1.14 (0.76–1.72)	1.12 (0.75–1.68)	Reference	0.99 (0.66–1.49)
	< 6.51	6.51 to <9.28	≥ 9.28	< 3.96	3.96 to <5.63	≥ 5.63
Stroke						
Cases/controls	80/91	191/190	103/93	85/91	162/190	127/93
Model 1	1.07 (0.71–1.64)	Reference	1.01 (0.63–1.62)	1.16 (0.75–1.79)	Reference	1.72 (1.14–2.60)
Model 2[a]	1.11 (0.73–1.74)	Reference	0.91 (0.55–1.48)	1.17 (0.75–1.82)	Reference	1.69 (1.11–2.58)
IS						
Cases/controls	61/69	147/154	87/72	63/68	131/152	101/75
Model 1	1.22 (0.76–1.98)	Reference	1.16 (0.67–2.01)	1.06 (0.64–1.77)	Reference	1.66 (1.05–2.65)
Model 2[a]	1.26 (0.78–2.06)	Reference	1.04 (0.59–1.83)	1.09 (0.65–1.84)	Reference	1.63 (1.02–2.62)
HS						
Cases/controls	19/22	44/36	16/21	22/23	31/38	26/18
Model 1	0.69 (0.23–2.04)	Reference	0.49 (0.15–1.58)	1.96 (0.66–5.82)	Reference	1.95 (0.65–5.82)
Model 2[a]	0.59 (0.19–1.88)	Reference	0.40 (0.12–1.49)	2.05 (0.67–6.26)	Reference	2.07 (0.66–6.47)

Note: Model 1 was adjusted for age, sex, BMI, smoking status, drinking status, family history of CVD, hypertension, hyperlipidemia, diabetes mellitus, physical activity (MET-hours/week), and use of aspirin.

Abbreviations: ACS, acute coronary syndrome; AMI, acute myocardial infarction; FX, coagulation factor X; FXI, coagulation factor XI; HS, hemorrhagic stroke; IS, ischemic stroke; UA, unstable angina.

[a]Model 2 was additionally adjusted for FX, platelet, and leukocyte except for covariates in model 1 for FXI analysis, and additionally adjusted for FXI, platelet, and leukocyte except for covariates in model 1 for FX analysis.

the risk of incident stroke with high FXI levels appeared to be more pronounced in subgroups of age more than 65 years old, female, hypertension, hyperlipidemia, and without diabetes mellitus, although these interactions were not significant.

FXI was a component of the early phase in the intrinsic pathway, and had been suggested to be a novel target for antithrombotic therapy.[13] Although previous studies had shown that FXI levels were associated with ischemic stroke.[31,32] However, these associations were based on case-control studies, which might be susceptible to survival bias, because samples were collected after diagnosis. Besides, a prospective study of prognosis including 576 participants after first ischemic stroke, found FXI activity was associated with higher risk of worse vascular outcomes (combined with stroke, myocardial infarction, and death) during 3 years of follow-up.[14] Anticoagulant and thrombolytic therapy after diagnosis might have potential confounding effects on vascular outcomes, although adjustment for some medications in that study.

Our study further extended previous finding in relatively healthy populations, showing higher risks for incident stroke and ischemic stroke in high FXI levels after multivariable adjustment during a median follow-up of 2.7 years. In consistent with our finding, a small nested case-control study including 495 white or African American participants (89 incident ischemic stroke cases) aged from 45 to 54 years, reported that FXI was associated with higher risk of incident ischemic stroke after multivariable adjustment.[10] Similar design with a large sample of our study might enhance statistic power.

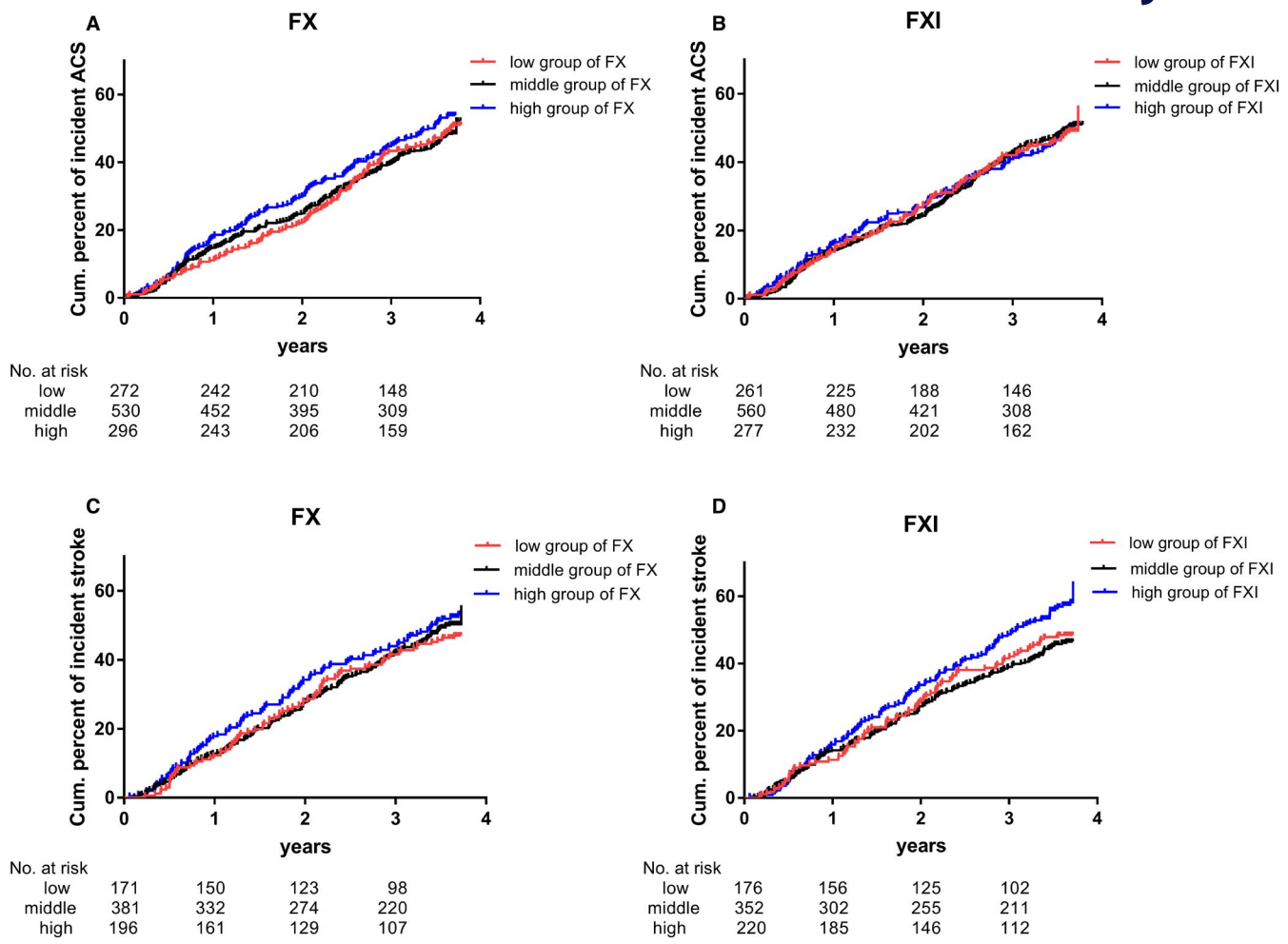

FIGURE 1 Kaplan-Meier cumulative percent of incident ACS and stroke according to FX and FXI levels, 2013–2016. Kaplan-Meier cumulative percent of incident ACS (A) and stroke (C) were shown according to FX levels, and Kaplan-Meier cumulative percent of incident ACS (B) and stroke (D) were shown according to FXI levels during the follow-up duration from 2013 to 2016 without adjustment. ACS, acute coronary syndrome; cum., cumulative; FX, factor X; FXI, factor XI

Other prospective studies were inconsistent with ours, showing that FXI was not associated with stroke or ischemic stroke in Whites and African Americans.[11,15,33] However, these prospective studies were all focused on Europeans and Americans, to our knowledge, we first performed the prospective study and found high FXI levels were associated with higher risk of incident stroke and ischemic stroke among Chinese. Thus, the population difference might be a possible explanation for the inconsistency. Moreover, samples in these prospective studies had been stored for a long time, even 20 years, which might decrease FXI levels and result in the null association. A study reported that FXI levels were stable in frozen plasma samples storage for 18 months and slightly decreased for 24 months.[34] Pre-experiment of a few samples in our study showed significant difference for 10 years storage at −80°C, not for 5 years, when compared with fresh plasma. Therefore, the long-term cryopreservation of samples might contribute to the inconsistency of associations between FXI levels and incident disease. Another rational explanation for the inconsistent results might be different exclusion criteria because we excluded the participants with liver cancer or had history of anticoagulant and antithrombotic medications, which might reduce possible confounding.

Platelet aggregation and inflammatory reaction might be involved in the pathogenesis of ACS and stroke, in addition to coagulation factors.[4,13,25] Previous studies also reported that platelet and leukocyte were associated with higher risk of CVD.[26,27] Of note, the associations of high FXI levels with incident stroke and ischemic stroke did not substantially change after additional adjustment for FX, platelet, and leukocyte in the present study. In line with our results, an inhibition of FXI (ISIS 416858) reduced FXI levels without an effect on bleeding in monkeys.[35] Moreover, FXI antisense oligonucleotides prevented thrombus formation on acutely ruptured atherosclerotic plaques with less severe inflammatory response in mice.[25] Although we adjusted for confounding factors more than previous studies, a limitation might exist because platelet was the count that might not reflect the activity and adhesion effect in the development of ACS and stroke.

However, we did not find any significant association of FXI levels with hemorrhagic stroke, which was in line with previous

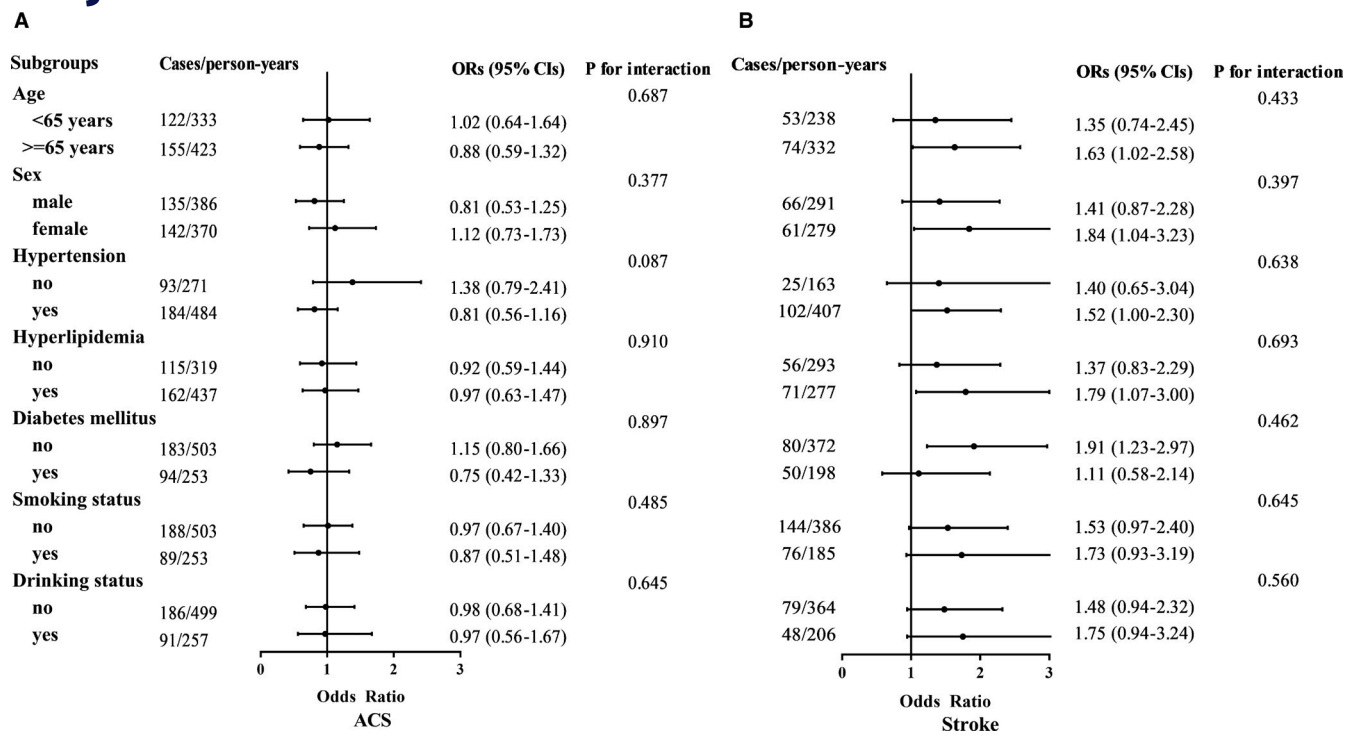

FIGURE 2 Adjusted odds ratios (ORs) for incident ACS and stroke in subgroups stratified by age, sex, hypertension, hyperlipidemia, diabetes mellitus, smoking status, and drinking status. Adjusted odds ratios (ORs) were only for high FXI levels with incident ACS (A) and stroke (B), and the reference groups were middle FXI levels. Adjusted for age, sex, BMI, smoking status, drinking status, family history of CVD, diabetes mellitus, hypertension, hyperlipidemia, physical activity (MET-hours/week), and use of aspirin (except the stratified variable). ACS, acute coronary syndrome; BMI, body mass index; CVC, cardiovascular disease; FXI, factor XI; MET, metabolic equivalent task hours

studies.[15,34,36] It was known that hemorrhagic stroke accounted for less than 20% of total stroke cases and had a higher mortality rate.[17] Less than 100 incident hemorrhagic stroke cases might limit the association. Moreover, although FXI deficiency could result in coagulopathy and hemorrhage,[37] participants in our study were general populations without FXI deficiency, which might contribute to the null association. Likewise, we did not observe a significant association between FXI levels and incident ACS, a fatal subtype of CHD. In line with us, a study including 368 incident CHD and 412 controls showed that the association of FXI levels with incident CHD was not significant.[11] On the contrary, FXI levels were related to myocardial infarction with a significant dose-response relationship in a case-control study.[38] As for these inconsistent findings, more prospective studies with large populations need to confirm.

FX was a central factor in the coagulation pathways,[9] but all associations of FX levels with ACS, stroke, and their subtypes were not significant in the study. Consistent with our results, two prospective studies also reported similar results of FX levels with CHD and ischemic stroke.[10,11] However, these prospective studies were based on relatively healthy populations with FX antigen levels, which might limit significant conclusions. On the contrary, a previous study observed that FX activity levels were associated with bleeding severity in rare bleeding disorders ($n = 489$) aged from 7 months to 95 years, although there may be heterogeneity.[12] Moreover, anticoagulation FX medication was used in the treatment of thrombus, which was used to inhibit FX activity.[5] Therefore, whether the associations of FX antigen levels with ACS and stroke were significant or not needs more prospective studies to evaluate.

We found the risk of incident stroke with high FXI levels appearing to be more pronounced in subgroups aged more than 65 years, female, hypertension, hyperlipidemia, and without diabetes mellitus. It seemed reasonable to suppose these traditional CVD risk factors might influence the effects of FXI on incident stroke. Inconsistent with several studies, females had higher FXI levels[16,39] and FXI levels were significantly associated with higher risk of stroke among females.[32] Possible explanations might be low estrogen levels, because most of female participants were in menopause in our study, although we did not measure estrogen levels. Estrogen had antiatherosclerotic and neuroprotective effects.[40] Furthermore, FXI levels were decreased during pregnancy, which was a physiological period with higher estrogen levels.[41] Likewise, FXI levels were associated with higher risk of ischemic stroke in dyslipidemia patients, which might be explained by the hypercoagulability of high cholesterol levels.[28] However, all interaction effects between FXI with these CVD risk factors were not significant, which might be due to the small sample size in certain subgroup limited these results. Thus, future studies need to verify our findings and further to elucidate the underlying effect modification.

Several strengths of our study were noteworthy. We prospectively explored the associations of FX and FXI levels with incident ACS, stroke, and their subtypes (acute myocardial infarction, unstable angina, ischemic stroke, and hemorrhagic stroke) in the nested

case-control study matched by age, sex, and sampling date, which minimized confounding effects of matched variables. Remarkably, we first found that the association of FXI levels with incident stroke was significant among Chinese, and the association might be attributed to ischemic not hemorrhagic stroke. Moreover, we evaluated the associations of FXI levels with incident stroke in subgroups stratified by traditional CVD risk factors, which contributed to elucidate the potential effects of FXI levels on incident stroke.

Nevertheless, our study also had several limitations. First, plasma FX and FXI levels were measured by enzyme-linked immunosorbent assay, indicating the levels were antigen levels, which might not reflect the bioactivity. However, a previous study had reported that the correlation coefficient was 0.667 between FXI activity levels and FXI antigen levels.[42] Moreover, FXI antigen levels were similar to FXI activity levels for risk of venous thrombosis.[39] Second, our findings were based on Chinese middle-aged and elderly people, which might be limited to younger and other populations. However, our results still had some public health implications. Third, although we found the risk of incident stroke with high FXI levels appearing to be more pronounced in some subgroups, the sample size in certain subgroup of strata may be small to limit definite conclusion of effect modification by those variables. Therefore, the possible effect modification needed to explore in larger sample size or special populations (e.g., hypertension, hyperlipidemia). Finally, the imputation of missing covariates might distort the results of the assessment, although the results of sensitivity analysis did not change substantially after we excluded participants with missing data. Moreover, residual confounding might exist, although we had considered a variety of confounders.

5 | CONCLUSION

In conclusion, the association of FXI with incident stroke might be attributed to ischemic but not hemorrhagic stroke and was robust to further adjustment for FX, platelet, and leukocyte. Moreover, FXI was not associated with incident ACS events and FX was not associated with incident ACS or stroke. Future studies need to validate our results and further to elucidate whether there is a causal relationship between FXI levels and incident ischemic stroke.

CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

Huiting Chen and Tangchun Wu designed the study. Huiting Chen carried out the statistical analyses, interpreted the data, and drafted the manuscript. Huiting Chen, Miaoyan Shen, Rundong Niu, Xuanwen Mu, Rong Peng, Qin Jiang, Yu Yuan, Hao Wang, Qiuhong Wang, Handong Yang, Xiaomin Zhang, Huan Guo, and Meian He contributed to the data collection and cleaning. All authors revised drafts critically for important intellectual content, and approved the final manuscript.

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REFERENCES

1. Vos TLim SS, Abbafati C, et al. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet*. 2020;396:1204-1222.
2. Zhou M, Wang H, Zeng X, et al. Mortality, morbidity, and risk factors in China and its provinces, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet*. 2019;394(10204):1145-1158.
3. Crea F, Libby P. Acute coronary syndromes: the way forward from mechanisms to precision treatment. *Circulation*. 2017;136(12):1155-1166.
4. Stoll G, Kleinschnitz C, Nieswandt B. Molecular mechanisms of thrombus formation in ischemic stroke: novel insights and targets for treatment. *Blood*. 2008;112:3555-3562.
5. Ruff CT, Giugliano RP, Braunwald E, et al. Comparison of the efficacy and safety of new oral anticoagulants with warfarin in patients with atrial fibrillation: a meta-analysis of randomised trials. *Lancet*. 2014;383:955-962.
6. Zhang H, Löwenberg EC, Crosby JR, et al. Inhibition of the intrinsic coagulation pathway factor XI by antisense oligonucleotides: a novel antithrombotic strategy with lowered bleeding risk. *Blood*. 2010;116:4684-4692.
7. Wang H, Rosendaal FR, Cushman M, Vlieg AVH. Procoagulant factor levels and risk of venous thrombosis in the elderly. *J Thromb Haemost*. 2021;19(1):186-193.
8. 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(10):696-701.
9. Borensztajn K, Peppelenbosch MP, Spek CA. Factor Xa: at the crossroads between coagulation and signaling in physiology and disease. *Trends Mol Med*. 2008;14(10):429-440.
10. Suri MFK, Yamagishi K, Aleksic N, Hannan PJ, Folsom AR. Novel hemostatic factor levels and risk of ischemic stroke: the atherosclerosis risk in communities (ARIC) study. *Cerebrovasc Dis*. 2010;29:497-502.
11. Yamagishi K, Aleksic N, Hannan PJ, Folsom AR. Coagulation factors II, V, IX, X, XI, and XII, plasminogen, and alpha-2 antiplasmin and risk of coronary heart disease. *J Atheroscler Thromb*. 2010;17:402-409.
12. Peyvandi F, Palla R, Menegatti M, et al. Coagulation factor activity and clinical bleeding severity in rare bleeding disorders: results from the European Network of Rare Bleeding Disorders. *J Thromb Haemost*. 2012;10(4):615-621.
13. Fredenburgh JC, Gross PL, Weitz JI. Emerging anticoagulant strategies. *Blood*. 2017;129:147-154.
14. Rohmann JL, Huo S, Sperber PS, et al. Coagulation factor XII, XI, and VIII activity levels and secondary events after first ischemic stroke. *J Thromb Haemost*. 2020;18:3316-3324.
15. Folsom AR, George KM, Appiah D. Lack of association of plasma factor XI with incident stroke and coronary heart disease: The atherosclerosis risk in communities (ARIC) study. *Atherosclerosis*. 2015;243:181-185.
16. Olson NC, Cushman M, Judd SE, et al. Associations of coagulation factors IX and XI levels with incident coronary heart disease and ischemic stroke: the REGARDS study. *J Thromb Haemost*. 2017;15:1086-1094.
17. Zakai NA, Olson NC, Judd SE, et al. Haemostasis biomarkers and risk of intracerebral haemorrhage in the reasons for geographic and racial differences in stroke study. *Thromb Haemost*. 2017;117:1808-1815.

18. Wang F, Zhu J, Yao P, et al. Cohort profile: the Dongfeng-Tongji cohort study of retired workers. *Int J Epidemiol*. 2013;42:731-740.
19. Richardson DB. An incidence density sampling program for nested case-control analyses. *Occup Environ Med*. 2004;61(12):e59.
20. Wright RS, Anderson JL, Adams CD, et al. 2011 ACCF/AHA focused update incorporated into the ACC/AHA 2007 guidelines for the management of patients with unstable angina/non-ST-elevation myocardial infarction: A report of the American College of Cardiology foundation/American Heart Association task force on practice guidelines developed in collaboration with the American Academy of Family Physicians, Society for Cardiovascular Angiography and Interventions, and the Society of Thoracic Surgeons. *J Am Coll Cardiol*. 2011;57:e215-e367.
21. Bassand JP, Hamm CW, Ardissino D, et al. Guidelines for the diagnosis and treatment of non-ST-segment elevation acute coronary syndromes. *Eur Heart J*. 2007;28:1598-1660.
22. Walker AE, Robins M, Weinfeld FD. The national survey of stroke. Clinical findings. *Stroke*. 1981;12:I13-I44.
23. Ainsworth BE, Haskell WL, Herrmann SD, et al. 2011 Compendium of physical activities: a second update of codes and MET values. *Med Sci Sports Exerc*. 2011;43(8):1575-1581.
24. Desquilbet L, Mariotti F. Dose-response analyses using restricted cubic spline functions in public health research. *Stat Med*. 2010;29(9):1037-1057.
25. Van Montfoort ML, Kuijpers MJE, Knaup VL, et al. Factor XI regulates pathological thrombus formation on acutely ruptured atherosclerotic plaques. *Arterioscler Thromb Vasc Biol*. 2014;34:1668-1673.
26. Welsh C, Welsh P, Mark PB, et al. Association of total and differential leukocyte counts with cardiovascular disease and mortality in the UK biobank. *Arterioscler Thromb Vasc Biol*. 2018;38:1415-1423.
27. Peng F, Li Z, Yi C, et al. Platelet index levels and cardiovascular mortality in incident peritoneal dialysis patients: a cohort study. *Platelets*. 2017;28:576-584.
28. Santamaría A, Oliver A, Borrell M, et al. Higher risk of ischaemic stroke associated with factor XI levels in dyslipidaemic patients. *Int J Clin Pract*. 2007;61(11):1819-1823.
29. Os HJAV, Wermer MJH, Rosendaal FR, Govers-Riemslag JW, Algra A, Siegerink BS. Intrinsic coagulation pathway, history of headache, and risk of ischemic stroke. *Stroke*. 2019;50(8):2181-2186.
30. Amitrano L, Guardascione MA, Brancaccio V, Balzano A. Coagulation disorders in liver disease. *Semin Liver Dis*. 2002;22:83-96.
31. Siegerink B, Govers-Riemslag JWP, Rosendaal FR, Ten Cate H, Algra A. Intrinsic coagulation activation and the risk of arterial thrombosis in young women: results from the risk of arterial thrombosis in relation to oral contraceptives (RATIO) case-control study. *Circulation*. 2010;122:1854-1861.
32. Siegerink B, Rosendaal FR, Algra A. Antigen levels of coagulation factor XII, coagulation factor XI and prekallikrein, and the risk of myocardial infarction and ischemic stroke in young women. *J Thromb Haemost*. 2014;12:606-613.
33. Appiah D, Fashanu OE, Heckbert SR, Cushman M, Psaty BM, Folsom AR. Relation of coagulation factor XI with incident coronary heart disease and stroke: the cardiovascular health study. *Blood Coagul Fibrinolysis*. 2017;28:389-392.
34. Woodhams B, Girardot O, Blanco MJ, Colesse G, Gourmelin Y. Stability of coagulation proteins in frozen plasma. *Blood Coagul Fibrinolysis*. 2001;12:229-236.
35. Younis HS, Crosby J, Huh J-I, et al. Antisense inhibition of coagulation factor XI prolongs APTT without increased bleeding risk in cynomolgus monkeys. *Blood*. 2012;119:2401-2408.
36. Gross BA, Jankowitz BT, Friedlander RM. Cerebral intraparenchymal hemorrhage: a review. *JAMA*. 2019;321:1295-1303.
37. Menegatti M, Peyvandi F. Treatment of rare factor deficiencies other than hemophilia. *Blood*. 2019;133:415-424.
38. Doggen CJM, Rosendaal FR, Meijers JCM. Levels of intrinsic coagulation factors and the risk of myocardial infarction among men: opposite and synergistic effects of factors XI and XII. *Blood*. 2006;108:4045-4051.
39. Folsom AR, Tang W, Roetker NS, Heckbert SR, Cushman M, Pankow JS. Prospective study of circulating factor XI and incident venous thromboembolism: the longitudinal investigation of thromboembolism etiology (LITE). *Am J Hematol*. 2015;90:1047-1051.
40. Pines A, Bornstein NM, Shapira I. Menopause and ischaemic stroke: basic, clinical and epidemiological considerations. The role of hormone replacement. *Hum Reprod Update*. 2002;8:161-168.
41. Phillips LL, Rosano L, Skrodelis V. Changes in factor XI (plasma thromboplastin antecedent) levels during pregnancy. *Am J Obstet Gynecol*. 1973;116:1114-1116.
42. Yang DT, Flanders MM, Kim H, Rodgers GM. Elevated factor XI activity levels are associated with an increased odds ratio for cerebrovascular events. *Am J Clin Pathol*. 2006;126:411-415.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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