

Original Paper

Factor XII as a Risk Marker for Hemorrhagic Stroke: A Prospective Cohort Study

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Keywords

Coagulation · Biomarkers · Intracranial hemorrhage · Cohort study · Cardiovascular disease

Abstract

Background: Coagulation factor XII (FXII) is involved in pathological thrombus formation and is a suggested target of anticoagulants. It is unclear whether FXII levels are correlated with cardiovascular risk factors and whether they are associated with myocardial infarction or ischemic or hemorrhagic stroke. The aim of this study was to investigate the correlation between FXII and cardiovascular risk factors in the general population. We also aimed to study the associations between FXII levels and future myocardial infarction and ischemic and hemorrhagic stroke. **Methods:** This prospective cohort study measured FXII levels in 1,852 randomly selected participants in a health survey performed in northern Sweden in 1994. Participants were followed until myocardial infarction, stroke, death, or until December 31, 2011. **Results:** During the median follow-up of 17.9 years, 165 individuals were diagnosed with myocardial infarction, 108 with ischemic stroke, and 30 with hemorrhagic stroke. There were weak correlations between FXII and body mass index, cholesterol, and hypertension. There was no association between FXII and myocardial infarction or ischemic stroke, neither in univariable Cox regression analysis nor after adjustment for age, sex, smoking, body mass index, cholesterol, hypertension, and diabetes. In univariable Cox regression analysis, the hazard ratio for the association between FXII levels and hemorrhagic stroke was 1.42 per SD

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(95% confidence interval: 0.99–2.05). In the multivariable model, higher levels of FXII were associated with increased risk of hemorrhagic stroke (hazard ratio 1.51 per SD; 95% confidence interval: 1.03–2.21). **Conclusion:** We found an independent association between FXII levels and the risk of hemorrhagic stroke, but not between FXII levels and ischemic stroke or myocardial infarction.

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Introduction

Myocardial infarction and stroke are leading causes of death worldwide [1]. There are biochemical markers that can be used to identify individuals with a high risk of cardiovascular events [2], and it has been suggested that factor XII (FXII), a protein involved in the intrinsic pathway of blood coagulation, could be a risk marker for thromboembolic disease [3–7]. Recent animal studies have suggested that FXII may have an important role in pathological thrombus formation [8–11] and that it is affected by the inflammatory process in acute stroke [12]. FXII inhibitors have been suggested as promising new antithrombotic agents [13], while epidemiological studies have reported that low levels of FXII are not associated with an increased risk of bleeding [13, 14]. In addition, animals treated with FXII inhibitors do not exhibit an increased bleeding risk, indicating that anticoagulants targeting FXII might be a safe alternative to current anticoagulant treatments [8–11]. Earlier research that investigated FXII as a possible risk marker for myocardial infarction and stroke reported contradicting results and rarely looked at data from the general population [3–7].

It is important to identify correlations between FXII levels and traditional cardiovascular risk factors in order to account for confounding factors, and previous studies have shown contradicting results with weak positive or absent associations with classical cardiovascular risk factors [15, 16].

FXII levels decrease in acute phase reactions [17]. Consequently, interpreting FXII levels in acute conditions is difficult, and prospective studies are needed to investigate FXII as a possible risk marker for disease. The American Atherosclerosis Risk in Communities (ARIC) study has examined the association between FXII levels and the risk of ischemic stroke and myocardial infarction and found no associations between FXII levels and future myocardial infarction or ischemic stroke. However, only people aged 45–64 years were included in the cohort [18, 19]. No studies have examined the relationship between FXII levels and hemorrhagic stroke, although sporadic case reports have linked FXII deficiency to intracranial bleeding [20, 21]. Ischemic and hemorrhagic stroke share many risk factors and it has been suggested that these conditions are two different manifestations of common underlying pathogenetic mechanisms [22]. The second Northwick Park Study (NPHS-II) found that lower levels of activated FXII-inhibitory complex seem to increase the risk of developing coronary heart disease and ischemic stroke in healthy middle-aged men [23].

Since the correlation between FXII and cardiovascular risk factors and the associations between FXII and future cardiovascular disease remain unclear, we investigated these relationships in a prospective population-based cohort study that evaluated the presence of cardiovascular risk factors in participants at baseline and involved 18 years of follow-up. The aim of this study was to investigate the correlation between FXII and cardiovascular risk factors and the associations between FXII levels and future myocardial infarction and ischemic and hemorrhagic stroke in the general population.

Methods

This prospective cohort study included the participants of a health survey that was conducted in northern Sweden in 1994. The participants were followed until myocardial infarction, stroke, death, or until the end of the study on December 31, 2011, whichever came first.

Study Population and Setting

The study population included participants in the 1994 Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) project in northern Sweden, a health survey initiated by the World Health Organization (WHO) with the aim of studying risk factors and trends in cardiovascular disease [24]. An age-stratified sample of 2,500 individuals aged 25–74 years, 1,250 women and 1,250 men, were invited to participate. Individuals were randomly selected among the residents of the counties of Västerbotten and Norrbotten, the two northernmost counties of Sweden, which had a combined population of 527,423 in 1994 [25]. Of the 2,500 people who were invited to participate, 1,921 attended the health survey and of these, 1,852 had a valid FXII measurement and were followed as a cohort until December 31, 2011. These 1,852 people comprised the study population. Information about dates of death was obtained from the population register at Statistics Sweden.

Event Registration

The National Patient Register and the Swedish Cause of Death Register were searched for ICD-9 and ICD-10 diagnosis codes for myocardial infarction (410, I21), ischemic stroke (434, I63), and hemorrhagic stroke (430, 431, I60, I61) for the years 1994 through 2011. Ten individuals with unspecified stroke (436, I64) were excluded from analyses concerning ischemic or hemorrhagic stroke. A participant flowchart is shown in Figure 1.

Measurements and Definitions

The baseline health survey was performed between January 1 and April 22, 1994, by dedicated teams that examined the participants at the nearest primary health care center. Participants underwent a health examination with blood samples and were asked to complete a questionnaire.

Data on smoking habits and antihypertensive and antidiabetic treatment were collected by a questionnaire. Smoking was defined as self-reported current or former smoking of at least 1 cigarette/day. Weight was recorded to the nearest 0.2 kg using a balance scale that was calibrated daily. Participants wore light clothing and no shoes while being weighed. Height without shoes was measured to the nearest centimeter. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters.

Blood pressure was measured twice using a Hawksley random zero sphygmomanometer with the participant in a sitting position after resting for 5 min. The mean of the 2 measurements was recorded. Hypertension was defined as systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg or the use of antihypertensive drugs during the 2 weeks preceding the health survey.

Blood samples were retrieved after a minimum of 4 h of fasting. Total serum cholesterol was analyzed using an enzymatic method (BM Monotest Cholesterol CHOD-PAP, Boehringer Mannheim GmbH, Mannheim, Germany). Venous blood samples for measuring fasting plasma glucose and 2-h oral glucose tolerance test (OGTT) plasma glucose were drawn into sodium fluoride tubes and analyzed using a hexokinase method (Boehringer Mannheim Automated Analysis for BM/Hitachi System 717, Mannheim, Germany). Diabetes was defined as fasting plasma glucose ≥ 7.0 mmol/L, 2-h OGTT plasma glucose ≥ 11.1 mmol/L or self-reported current use of antidiabetic medication.

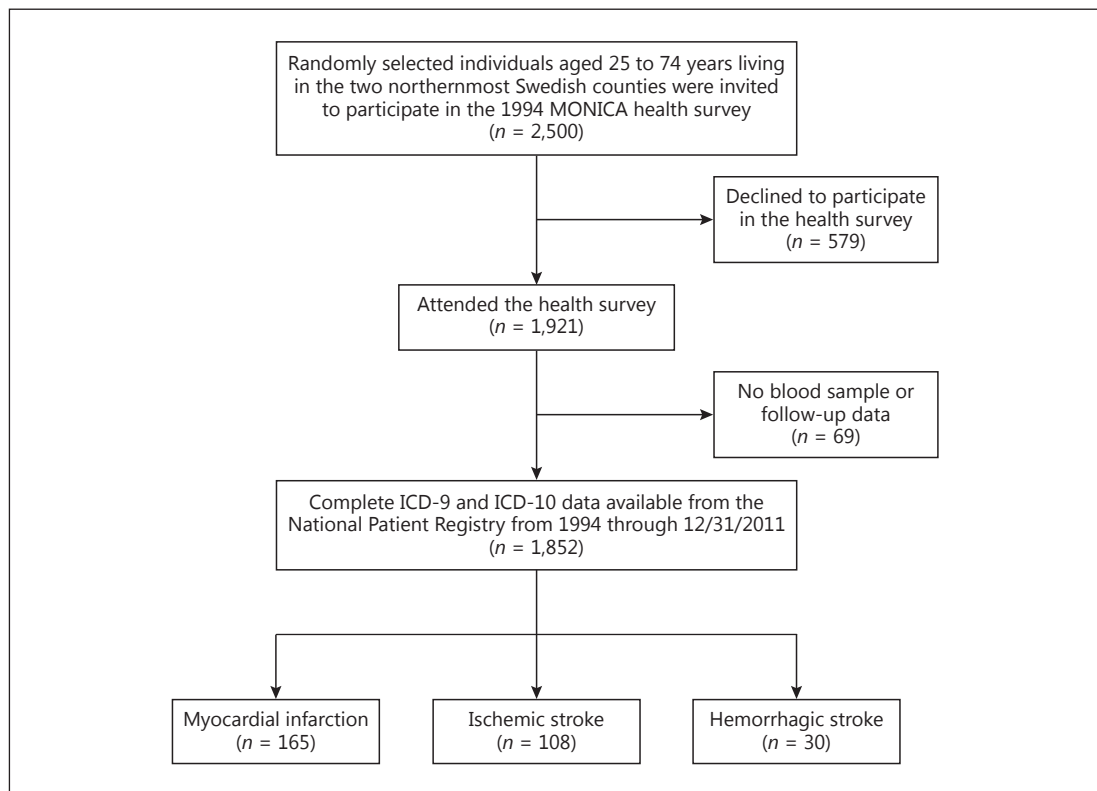


Fig. 1. Participant flow diagram.

Blood samples were collected for future research. Erythrocytes and plasma were separated by centrifugation at 1,500 *g* for 15 min, and plasma was divided into aliquots and stored at -80°C until analysis. For FXII analysis, blood was collected in EDTA Venoject tubes (Terumo, Leuven, Belgium). Zymogen FXII levels were determined using a chromogenic substrate assay described by Tankersley et al. [26] which is based on dextran sulfate-induced activation of prekallikrein to kallikrein by activated FXII. The rate of kallikrein generation over time, mediated by activated FXII, is recorded by a chromogenic substrate sensitive to kallikrein, S-2302, and is proportional to the original FXII zymogen concentration in the participant's plasma sample. Reagents were prepared as described by Tankersley et al. [26] except that the prekallikrein purification was scaled up to allow the processing of 1.5 L of human plasma and the analyses were performed on a Hitachi 911 automatic analyzer. Pooled plasma from 20 healthy individuals (laboratory staff) was used to construct standard curves, and calibration to international units per milliliter was performed using a WHO calibrator plasma. All samples were analyzed at the same location and at the same time (1997). Laboratory staff had no knowledge of event status.

Missing Values

The date of the health survey was missing in 4 participants, who were assigned the median date of the health survey. Questionnaire data on smoking habits, antihypertensive treatment, and antidiabetic treatment were missing in 4, 6, and 6 participants, respectively. Of the 6 patients with missing antidiabetic treatment data, 2 had valid OGTT results. BMI data were missing in 4 participants. Blood pressure measurements were missing in 1 participant. None of the participants had missing cholesterol levels data. Participants with a missing value for one variable were excluded from all analyses that included that variable.

Table 1. Baseline characteristics of the study population ($n = 1,852$)

Characteristics	
Age, years	50 (38–62)
Female, n	938 (50.6)
Smoker ^a , n	923 (49.9)
Median BMI, kg/m ²	25.5 (23.1–28.3)
Median cholesterol, mmol/L	6.0 (5.2–6.9)
Hypertension ^b , n	710 (38.4)
Diabetes ^c , n	84 (4.5)
Median factor XII, IU/mL	1.12 (0.92–1.32)

Values in parentheses are 1st–3rd quartiles or percentages. BMI, body mass index. ^a Current or former smoker. ^b Systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, or antihypertensive treatment. ^c Pharmacologically treated diabetes, fasting plasma glucose ≥ 7.0 mmol/L, or 2-h oral glucose tolerance test plasma glucose ≥ 11.1 mmol/L.

Statistical Methods

The number of observations, percentages, and the median and interquartile range were calculated for the baseline characteristics. The Spearman rank order correlation was used for testing the correlation between FXII levels and traditional cardiovascular risk factors. A two-sided p value of <0.05 was considered significant. The normality of distribution was tested by visual inspection of Q-Q plots. All variables were considered normally distributed. The assumption of proportional hazards was verified graphically using Kaplan-Meier survival curves. Myocardial infarction and ischemic and hemorrhagic stroke were analyzed separately, and only the first occurrence of each outcome during the study period was considered. The time-to-event was defined as the time from the health survey date to the diagnosis date.

Cox proportional hazard regression was used to investigate the associations between FXII levels, traditional cardiovascular risk factors, and the outcomes in univariable and multivariable analyses. All variables included in the univariable analyses were included in the multivariable model. In order to facilitate comparisons between hazard ratios (HRs), all continuous variables were divided by their standard deviations (SDs) before being entered in the model. FXII was also analyzed as a categorical variable using tertiles to enable the detection of a stepwise change in risk. All calculations were performed with SPSS software version 22.0 (Chicago, IL, USA).

The study was approved by the Regional Ethics Review Board, Umeå, Sweden. All study participants provided informed written consent.

Results

The baseline characteristics of the 1,852 participants are shown in Table 1. The median participant age was 50 years, and 51% were women. The median duration of follow-up was 17.9 years, with a total follow-up time of 30,372 patient-years. The median FXII level was 1.12 IU/mL. Correlations between FXII levels and age, sex, smoking, BMI, cholesterol, hypertension, and diabetes are shown in Table 2. High FXII levels were correlated with high BMI, high cholesterol levels, and hypertension. The associations between FXII levels and incident myocardial infarction, ischemic stroke, and hemorrhagic stroke are shown in Table 3 and in Figure 2.

Table 2. Spearman correlation coefficients between factor XII and cardiovascular risk factors

	Age	Sex	Smoking ^a	BMI	Cholesterol	Hypertension ^b	Diabetes ^c
Factor XII	-0.02	<0.01	-0.03	0.07**	0.13**	0.06*	0.04

BMI, body mass index. * $p < 0.05$; ** $p < 0.001$. ^a Current or former smoker. ^b Systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, or antihypertensive treatment. ^c Pharmacologically treated diabetes, fasting plasma glucose ≥ 7.0 mmol/L, or 2-h oral glucose tolerance test plasma glucose ≥ 11.1 mmol/L.

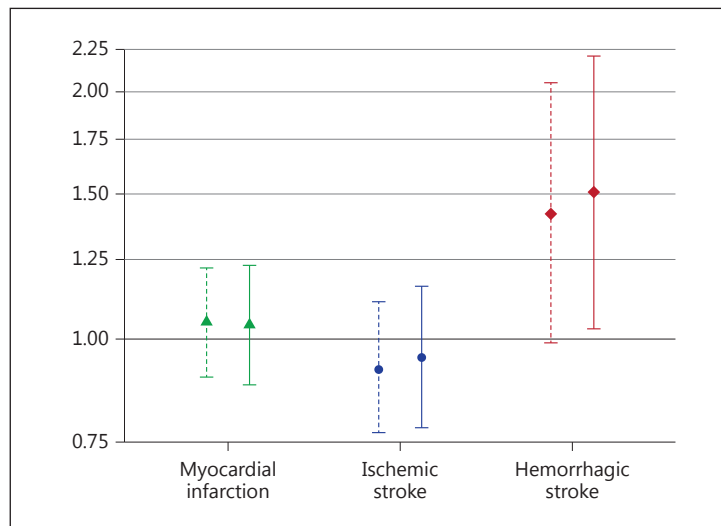
Table 3. Hazard ratios with 95% confidence intervals showing the associations between cardiovascular risk factors, factor XII, and myocardial infarction, ischemic and hemorrhagic stroke

	Myocardial infarction (n = 165)		Ischemic stroke (n = 108)		Hemorrhagic stroke (n = 30)	
	univariable	multivariable	univariable	multivariable	univariable	multivariable
Age by 10-year groups	2.27 (1.96–2.63)*	2.06 (1.75–2.43)*	2.97 (2.41–3.65)*	2.76 (2.20–3.47)*	1.76 (1.30–2.37)*	1.79 (1.25–2.56)*
Sex/male	2.37 (1.71–3.29)*	2.54 (1.82–3.56)*	1.60 (1.09–2.35)*	1.54 (1.04–2.29)*	0.95 (0.46–1.95)	0.87 (0.42–1.81)
Smoking ^a	1.39 (1.02–1.90)*	1.45 (1.06–1.98)*	1.09 (0.75–1.59)	1.26 (0.85–1.85)	1.81 (0.86–3.80)	2.07 (0.97–4.39)
BMI per SD	1.39 (1.23–1.57)*	1.17 (1.00–1.37)*	1.25 (1.06–1.48)*	0.99 (0.81–1.21)	1.14 (0.82–1.60)	0.95 (0.65–1.37)
Cholesterol per SD	1.62 (1.41–1.86)*	1.28 (1.09–1.51)*	1.33 (1.11–1.59)*	0.91 (0.73–1.12)	1.16 (0.82–1.64)	0.81 (0.54–1.21)
Hypertension ^b	3.63 (2.63–5.01)*	1.35 (0.95–1.94)	4.95 (3.25–7.54)*	1.90 (1.20–3.02)*	2.72 (1.31–5.65)*	1.51 (0.65–3.54)
Diabetes ^c	3.70 (2.32–5.90)*	1.86 (1.15–3.02)*	3.01 (1.61–5.62)*	1.26 (0.67–2.39)	2.79 (0.84–9.19)	1.46 (0.43–5.01)
FXII per SD	1.05 (0.90–1.22)	1.04 (0.88–1.23)	0.92 (0.77–1.11)	0.95 (0.78–1.16)	1.42 (0.99–2.05)	1.51 (1.03–2.21)*

Associations are presented as univariable and multivariable hazard ratios. SD, standard deviation; BMI, body mass index; FXII, factor XII. * $p < 0.05$.

^a Current or former smoker. ^b Systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, or antihypertensive treatment. ^c Pharmacologically treated diabetes, fasting plasma glucose ≥ 7.0 mmol/l, or 2-h oral glucose tolerance test plasma glucose ≥ 11.1 mmol/l.

Fig. 2. Associations between levels of factor XII per standard deviation and risk of myocardial infarction, ischemic stroke, and hemorrhagic stroke. Associations are presented as hazard ratios with 95% confidence intervals. Results from univariable analyses are presented as dashed lines and results from multivariable analyses are presented as solid lines.



Myocardial Infarction

During follow-up, 165 individuals were diagnosed with myocardial infarction after a median time-to-event of 9.5 years. Univariable Cox proportional hazard regression analysis showed no association between FXII levels and myocardial infarction (HR 1.05 per SD; 95% CI: 0.90–1.22). There was no association between tertiles of FXII and myocardial infarction (data not shown). A multivariable Cox regression model adjusted for age, sex, smoking, BMI, cholesterol, hypertension, and diabetes showed no association between FXII levels and myocardial infarction (HR 1.04 per SD; 95% CI: 0.88–1.23).

Ischemic Stroke

During the study period, 108 individuals were diagnosed with ischemic stroke after a median time-to-event of 11.4 years. Univariable Cox proportional hazard regression analysis showed no association between FXII levels and ischemic stroke (HR 0.92 per SD; 95% CI: 0.77–1.11). There was no association between tertiles of FXII and ischemic stroke (data not shown). In a multivariable Cox regression model adjusted for age, sex, smoking, BMI, cholesterol, hypertension, and diabetes, there was no association between FXII levels and ischemic stroke (HR 0.95 per SD; 95% CI: 0.78–1.16).

Hemorrhagic Stroke

During the study period, 30 individuals were diagnosed with hemorrhagic stroke after a median time-to-event of 11.1 years. Of these individuals, 20 had intracerebral bleeding and 10 had subarachnoid bleeding. In univariable Cox regression analysis, the HR for the association between FXII levels and hemorrhagic stroke was 1.42 per SD (95% CI: 0.99–2.05). In a multivariable model adjusted for age, sex, smoking, BMI, cholesterol, hypertension, and diabetes, higher levels of FXII were significantly associated with increased risk of hemorrhagic stroke (HR 1.51 per SD; 95% CI: 1.03–2.21). When the relationship between tertiles of FXII and hemorrhagic stroke was investigated using univariable Cox regression, the HR was 2.10 (95% CI: 0.79–5.59) for the 2nd tertile and 2.00 (95% CI: 0.75–5.33) for the 3rd tertile using the lowest tertile of FXII as the reference category. When analyzing intracerebral bleeding and subarachnoid bleeding separately, the adjusted HR for the association between FXII and intracerebral bleeding was 1.50 per SD of FXII (95% CI: 0.95–2.34), and for subarachnoid bleeding, the HR was 1.30 per SD of FXII (95% CI: 0.69–2.44).

Discussion

This prospective cohort study found a significant association between baseline FXII levels and the risk of hemorrhagic stroke in a model adjusted for age, sex, smoking, BMI, cholesterol, hypertension, and diabetes. In contrast, FXII levels did not predict myocardial infarction or ischemic stroke. There were weak (but in a few factors significant) correlations between FXII levels and cardiovascular risk factors in our study, which is in line with previous findings [18, 27].

In our study, there was no association between FXII levels and myocardial infarction or ischemic stroke. The prospective ARIC study found no significant differences in FXII levels between individuals with and without cardiovascular disease [18, 19]. A case-control study of young women with ischemic stroke and healthy controls found no differences in FXII levels between cases and controls [28].

In our study, we found an association between high FXII levels and increased risk of hemorrhagic stroke. Both epidemiological studies and animal studies have demonstrated that low FXII levels are not associated with an increased risk of bleeding [9, 14]. An associ-

ation between high FXII levels and increased risk of hemorrhagic stroke or other bleeding conditions has not been described previously. While there have been observational studies of the putative association between FXII levels and thromboembolic disease, to our knowledge there have been no prospective population-based studies that investigated FXII levels as a possible risk marker for hemorrhagic stroke. However, decreased levels of other coagulation factors, such as von Willebrand factor, are associated with future hemorrhagic stroke [29]. Both intracerebral hemorrhage and subarachnoid bleeding are conditions with a high mortality [30, 31]. Using a biomarker such as FXII to find high-risk groups in which preventive strategies could be implemented could help lower the number of deaths attributable to hemorrhagic stroke.

Hypertension is the most important risk factor for intracerebral hemorrhage. Chronic hypertension induces injuries and inflammation in the arterial wall which over time make the vessel fragile. Ultimately, the vessel may rupture and intracerebral hemorrhage ensues [32, 33]. The association we found between high FXII levels and hemorrhagic stroke may reflect the presence of a vessel wall injury in the cerebral arteries making them prone to rupture rather than occlusion. Since the median follow-up time in our study was 18 years, elevated levels of FXII could reflect a low-grade vessel wall injury over long time.

Strengths

The strengths of this study include its population-based study design, the inclusion of a random sample of the population with a wide range of ages (25–74 years), and the long follow-up period (median follow-up time of 18 years). The participation rate in the Northern Sweden MONICA survey was 80% in 1994. Telephone interviews of nonparticipants were conducted to assess possible nonrespondent bias in the MONICA Northern Sweden survey, and the overall nonrespondent bias was low [34]. Therefore, we consider the study population to be a representative sample of the population in northern Sweden. With 1,852 participants, it is unlikely that we missed a clinically relevant correlation between FXII and cardiovascular risk factors. The myocardial infarction and stroke diagnoses were collected from the National Patient Register. The validity of National Patient Register diagnoses of stroke and myocardial infarction, which were used as outcomes in this study, is high [35, 36].

Limitations

As there were a limited number of events, especially for hemorrhagic stroke, the results of our study should be considered as hypothesis generating. Due to the limited number of bleeding events, we evaluated intracerebral and subarachnoid bleeding as a combined event-hemorrhagic stroke. Our results indicate an association between FXII levels and both intracerebral bleeding and subarachnoid bleeding. As the pathophysiology of these diseases differs, forthcoming studies should investigate the association between FXII levels and intracerebral and subarachnoid bleeding separately. Due to the limited number of events, it is possible that we failed to detect weak associations between FXII levels, myocardial infarction and ischemic stroke. However, this seems unlikely since the HRs for the associations between FXII levels, myocardial infarction and ischemic stroke were very close to 1.0. It would also have been interesting to analyze different subtypes of ischemic stroke separately, as other studies have shown that levels of other biomarkers differ across subtypes [37–39]. Another limitation is the inclusion of inhabitants from just one geographical area. Notably, the population levels of FXII vary between countries and ethnicities, perhaps due to the strong association between FXII levels and FXII genetic polymorphisms [40, 41]. FXII levels were measured at a single time point, which may have led to an underestimation of the association between the risk marker and the outcome [42]. However, a previous study showed good within-person correlation of FXII antigen levels measured at baseline and 5 years later [14].

In conclusion, there were no relevant correlations between FXII levels and cardiovascular risk factors. There were no associations between FXII levels and the risk of myocardial infarction or ischemic stroke. FXII levels predicted hemorrhagic stroke in a model that was adjusted for sex, age, and traditional cardiovascular risk factors. However, before suggesting high FXII as a novel risk marker for hemorrhagic stroke, further studies are needed to verify this association.

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Author Contributions

K. Johansson, M. Lind, L. Johansson, and J.-H. Jansson were responsible for the study concept and design. I. Bylesjö proposed the study of FXII levels in this cohort and took part in the planning of the study. T.K. Nilsson prepared the reagent and performed the FXII assays. K. Johansson was responsible for statistical analysis, interpretation of the data, and drafting of the manuscript. M. Lind, L. Johansson, J.-H. Jansson, T.K. Nilsson, M. Eliasson, and S. Söderberg were responsible for critical revision of the intellectual content of the manuscript. M. Eliasson was responsible for clinical data acquisition and blood sampling. S. Söderberg was responsible for event registration. All authors approved the final version of the manuscript.

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