An Association Between Clotting Factor VII and Carotid Intima-Media Thickness

The CARDIA Study

David Green, MD, PhD; Nancy Foiles, MT; Cheeling Chan, MS; Joseph Kang, PhD; Pamela J. Schreiner, PhD; Kiang Liu, PhD

Background and Purpose—To investigate associations of procoagulants (factor VII [FVII], FVIII, von Willebrand factor) with subclinical atherosclerosis, we examined participants in the Coronary Artery Risk Development in Young Adults (CARDIA) study.

Methods—Clotting factor assays were performed in 1254 participants 23 to 37 years of age (baseline) and repeated at ages 38 through 50 (follow-up). Carotid intima-media thickness (IMT) was measured at follow-up.

Results—Baseline levels of procoagulants (%), mean (SD) were: FVII, 76 (18); FVIII, 102 (38); and von Willebrand factor, 108 (47). At follow-up, all had increased by 40% to 55%. After age adjustment, mean common carotid IMT increased from the lowest to the highest tertile of FVII in the total group (0.787 to 0.801; \( P = 0.007 \)), in whites (0.772 to 0.790; \( P = 0.002 \)), and in men (0.807 to 0.827; \( P = 0.015 \)). All associations were attenuated by multivariable adjustment. However, participants with FVII values in the highest tertile at one or both examinations, compared with those in the lowest tertile, had greater common carotid IMT after age and multivariable adjustment (0.806 versus 0.778; \( P < 0.05 \)). Baseline FVIII was associated with greater internal carotid IMT in the total group, in whites, and in women after age adjustment but not multivariable adjustment. No associations were seen for von Willebrand factor.

Conclusions—FVII is associated with common carotid IMT in young adults, but the strength of the association is modified by other cardiovascular disease risk factors, such as body mass index. FVIII is associated with internal carotid IMT only in age-adjusted analyses, and no associations were observed for von Willebrand factor. (Stroke. 2010;41:1417-1422.)

Key Words: factor VII ■ carotid thickening ■ atherosclerosis ■ factor VIII

Ischemic stroke is characterized by thrombotic occlusion of cerebral vessels, but whether elevated concentrations of specific coagulant proteins are associated with stroke risk is unclear. The question is important because if such relationships were established, measuring procoagulant protein concentrations might provide prognostic as well as therapeutic approaches to stroke management. To identify associations of procoagulants with the development of vascular disease, we measured hemostatic factors in healthy young adults, 25 to 37 years of age. Thirteen years later, we repeated the hemostatic factor measurements and searched for evidence of subclinical atherosclerosis. We then determined whether there were associations between the clotting factor levels at either time interval and the presence of subclinical cardiovascular disease (CVD).

Methods

Coronary Artery Risk Development in Young Adults Study

Participants were from the Coronary Artery Risk Development in Young Adults (CARDIA) study, a multicenter longitudinal study designed to investigate the evolution of CVD risk factors and subclinical atherosclerosis. Details of the design have been published previously.1 All data collection technicians were centrally trained and certified, and the CARDIA coordinating center and the CARDIA quality control committee monitored data collection throughout the study. The study was approved by the institutional review board at each field center, and informed consent was obtained from each participant at each examination. Participants’ age, race, gender, and cigarette use were assessed by questionnaire. Anthropometric variables included height and weight, body mass index (BMI), and blood pressure. Height and weight were measured using a balance beam scale and a vertical ruler, respectively, with the participant wearing light clothing and no shoes. BMI was calculated as the weight (kg) divided by the height in meters squared (m²). The resting blood pressure was measured in the right arm using a random-zero sphygmomanometer at baseline and with an automated Omron device at follow-up. Hypertension was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or current use of antihypertensive medication. Biochemical variables included total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein cholesterol, triglycerides, C-reactive protein (CRP), fasting glucose, and insulin. Diabetes was defined as fasting glucose ≥7.0 mmol/L or current use of diabetic medications. In 1992 and 1993, as part of the year-7 CARDIA examination, hemostatic...
The coefficients of variation of the assays were determined by using split samples derived from the study participants. At baseline, factor VII (FVII) and FVIII coagulant activities were measured in EDTA plasma by an immunoturbidimetric method (VisuLize FVIII Antigen Kit; Affinity Biologicals), which uses a double-antibody ELISA. FVIII antigen was assayed in EDTA plasma using the Asserachrom VII-Ag reagents and ELISA obtained from Diagnostica Stago. FVII antigen was assayed in EDTA plasma using the MINANALYZE procedure.

Managing Missing Data
To assess sensitivity because of missing information in some covariates and outcomes of interest, we performed analyses using multiple imputation. Although the missing proportion was \( \approx 23\% \) of the original sample (1619; Figure 1), the imputed data sets resulted in consistent point and interval estimates compared with the estimates from unimputed data sets (data sets that exclude people who have partially observed information). The implementation of multiple imputation in this article was based on the procedure described by Raghunathan et al using IVEware software, and the standard errors adjusted for imputation were estimated using the SAS MIANALYZE procedure.
For the longitudinal analyses, the independent variables were the baseline procoagulants, and the outcome variables were follow-up IMT. The covariates were baseline age, race, gender, BMI, smoking, education, center, systolic blood pressure, diabetes, antihypertensive medication use, total and HDL cholesterol, and CRP level. For the cross-sectional analyses, the independent variables were follow-up procoagulants, and the outcome variables were follow-up IMT. The covariates were follow-up age, race, gender, BMI, smoking, education, center, systolic blood pressure, diabetes, antihypertensive medication use, total and HDL cholesterol, and CRP level. For the hemostatic group analyses, the covariates were baseline age, BMI, smoking, education, center, systolic blood pressure, diabetes, antihypertensive medication use, total and HDL cholesterol, and CRP level. All analyses were conducted using SAS statistical software (version 9.2; SAS Institute Inc).

Results
Table 1 shows the baseline characteristics of the study participants according to gender–race group. Blacks were younger than whites, and black women had a greater BMI. Black participants had less education and more were current smokers. Black women tended to have higher systolic blood pressures than white women, and black men had higher HDL cholesterol than white men. CRP levels were higher in blacks than whites. FVIII and vWF levels were higher in blacks than whites, and the mean values of all hemostatic factors were higher at follow-up (percentage increase: FVII, 48%; FVIII, 55%; and vWF, 40%).

Factor VII
Longitudinal associations between IMT and FVII were evaluated by examining baseline factor levels and IMT recorded at follow-up. After adjustment for age, race, and gender, there was a significant trend \( (P=0.007) \) of higher FVII levels with CC-IMT but not with IC-IMT (Figure 2A). On further analysis, the association of CC-IMT with FVII was restricted to whites \( (P<0.002) \) and men \( (P=0.015) \), but the trend was no longer apparent after multivariable adjustment for baseline age, BMI, smoking, education, field center, systolic blood pressure, diabetes, antihypertensive medication use, total and HDL cholesterol, and CRP level (data not shown). In regression analyses that included potential covariates one at a time into the age-, gender- and race-adjusted model, confounding was found to be primarily attributable to BMI.

Figure 2B displays a cross-sectional analysis of follow-up FVII levels and CC-IMT. Similar to the longitudinal data, significant trends were observed in the age, race-, and gender-adjusted model between FVII and CC-IMT in the total group \( (P=0.002) \), in whites \( (P=0.001) \), and men \( (P=0.007) \), but not with IC-IMT. Again, significance was lost on multivariable adjustment.

Next, we performed an analysis to examine whether having a FVII in the highest tertile at baseline, follow-up, or both would be associated with IMT. Significant associations were noted after adjustment for age, race, and gender between CC-IMT and participants having ≥1 FVII levels in the highest tertile in the total group and in whites \( (P<0.05) \) but not in blacks (Figure 3). Figure 3 also shows that significance was retained after imputation analysis for missing data. On multivariable analysis, significance was retained for whites \( (P<0.05) \) but not for the total group. No associations were observed for IC-IMT.

Factor VIII
With respect to FVIII, there was a significant association between baseline FVIII and IC-IMT for the total group \( (n=1083) \) after age, race, and gender adjustment \( (P=0.015) \) but not after multivariable adjustment. There was also a trend for an association between IC-IMT and whites \( (n=706; P=0.054) \) and women \( (n=586; P=0.034) \), but significance was not retained after multivariable adjustment. No significant associations were observed between follow-up FVIII and CC-IMT on cross-sectional analyses or after grouping participants according to whether FVIII was in the lowest or highest tertiles at baseline or follow-up.

Table 1. Characteristics of Study Participants According to Gender–Race Group, the CARDIA Study, 1992–2006*

<table>
<thead>
<tr>
<th></th>
<th>Total n=1254</th>
<th>Black (n=189)</th>
<th>White (n=388)</th>
<th>Black (n=278)</th>
<th>White (n=399)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>32.4 (3.5)</td>
<td>31.6 (3.7)†</td>
<td>32.6 (3.3)</td>
<td>31.7 (3.9)†</td>
<td>33.0 (3.2)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.5 (5.7)</td>
<td>26.8 (5.3)</td>
<td>26.2 (4.1)</td>
<td>28.8 (7.5)†</td>
<td>25.1 (5.4)</td>
</tr>
<tr>
<td>Education (y)</td>
<td>14.6 (2.5)</td>
<td>13.2 (2.2)†</td>
<td>15.4 (2.6)</td>
<td>13.3 (1.8)†</td>
<td>15.4 (2.4)</td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>28.4</td>
<td>43.9†</td>
<td>20.4</td>
<td>37.0†</td>
<td>22.8</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>1.2</td>
<td>1.6</td>
<td>0.8</td>
<td>0.7</td>
<td>1.7</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>108 (11)</td>
<td>112 (10)‡</td>
<td>110 (10)</td>
<td>109(13)†</td>
<td>103 (9)</td>
</tr>
<tr>
<td>Antihypertensive medication (%)</td>
<td>1.3</td>
<td>0.5</td>
<td>1.3</td>
<td>1.8</td>
<td>1.2</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)§</td>
<td>177 (33)</td>
<td>177 (36)†</td>
<td>180 (36)</td>
<td>174 (31)</td>
<td>176 (30)</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)§</td>
<td>52 (14)</td>
<td>52 (15)†</td>
<td>45 (11)</td>
<td>57 (16)</td>
<td>56 (13)</td>
</tr>
<tr>
<td>CRP (ug/mL), median (IQR)</td>
<td>1.01 (0.45 to 2.78)</td>
<td>1.14 (0.51 to 2.55)†</td>
<td>0.77 (0.38 to 1.84)</td>
<td>1.76 (0.57 to 4.95)†</td>
<td>1.06 (0.36 to 2.95)</td>
</tr>
<tr>
<td>FVII (%)</td>
<td>76 (18)</td>
<td>74 (18)</td>
<td>75 (18)</td>
<td>76 (16)</td>
<td>77 (18)</td>
</tr>
<tr>
<td>FVIII (%)</td>
<td>102 (38)</td>
<td>111 (44)‡</td>
<td>93 (30)</td>
<td>112 (44)†</td>
<td>99 (35)</td>
</tr>
<tr>
<td>vWF (%)</td>
<td>108 (47)</td>
<td>125 (59)†</td>
<td>97 (35)</td>
<td>120 (57)†</td>
<td>103 (36)</td>
</tr>
</tbody>
</table>

*Data shown are means (SD) unless otherwise indicated. All variables were measured at baseline. Diabetes was defined as fasting glucose >7.0 mmol/L or current use of diabetic medications. IQR indicates interquartile range. †P<0.001; ‡P<0.05 compared with whites within gender. §To convert to SI units, multiply by 0.0259.
Von Willebrand Factor

No significant associations were observed between vWF and IMT at either examination or after grouping participants according to whether vWF was in the lowest or highest tertiles at baseline or follow-up.

Discussion

A consistent finding of this study is the positive association of FVII with CC-IMT. This association was found whether FVII was measured when participants were 25 to 37 years of age or 38 to 50 years of age. Furthermore, it was observed in
participants having ≥1 FVII levels in the highest tertile at either age and was highly significant for whites and men. These associations between FVII and IMT were observed in age-/gender-/race-adjusted analyses but were attenuated after adjustment for BMI, total cholesterol, and HDL cholesterol, factors shown previously to be positively associated with FVII in young adults. Thus, FVII is not an independent risk factor for carotid thickness.

Nearly 30 years ago, the Northwick Park Heart Study described a strong association of FVII, FVIII, and fibrinogen with cardiovascular death in men 40 to 64 years of age at study inception. However, in a subsequent study, they found associations were weaker: hazard ratios for stroke were 1.36 (95% CI, 1.09 to 1.69) for fibrinogen and 1.07 (95% CI, 1.01 to 1.12) for FVII (Table 2). The Atherosclerosis Risk in Communities study measured hemostatic factors in nearly 15 000 participants 45 to 64 years of age initially free of CVD, followed for up to 9 years. The adjusted relative risk for occurrence of ischemic stroke in those in the highest versus lowest fourth of clotting factor were 1.03 (95% CI, 0.88 to 1.21) for FVII, 1.93 (95% CI, 1.2 to 3.1) for FVIII, 1.71 (95% CI, 1.1 to 2.7) for vWF, and 1.26 (95% CI, 0.8 to 2.0) for fibrinogen. The Cardiovascular Health Study, an examination of nearly 6000 persons 65 years of age, found associations with stroke or transient ischemic attack in women for FVIII (relative risk, 1.4), and in men for fibrinogen (relative risk, 1.3). Although the initial examination of study data did not show a consistent association of FVII with stroke, on follow-up, a significant adjusted hazard ratio of 1.6 (95% CI, 1.2 to 2.2) was reported. They also observed that 6 single-nucleotide polymorphisms were associated with strokes, and 4 of these were in FVII-related genes.

Elevated levels of FVII have been reported in persons with carotid wall thickening and in men with coronary events. FVII is increased in women with coronary atheroma, and common FVII haplotypes contribute to the risk of myocardial infarction in women. However, the Glostrup study of 695 60-year-old Danish men and women did not find an association of FVII and carotid IMT. This suggests that the effect of FVII on atherogenesis is complex and influenced by a variety of risk factors. Our findings that the association of FVII with IMT is affected by BMI are in agreement with this view.

FVII is activated by tissue factor, and the FVIIa–tissue factor complex activates both intrinsic (FVIII, FIX) and extrinsic (FX) coagulation pathways. Raised FVII concentrations enhance thrombin generation and fibrin formation and contribute to vascular occlusion. In contrast to our observations regarding FVII, we found few associations of FVIII with carotid IMT, and none remained significant after multivariable adjustment. There were also no associations of vWF with IMT. Although increased FVIII has been associated with arterial vascular events before 50 years of age, such persons usually have a family history of elevated FVIII or vascular events. A possible explanation for why FVII, but not FVIII or vWF, is associated with subclinical atherosclerosis relates to the noncoagulant activity of FVII; it binds to tissue factor, and the complex induces smooth muscle cell proliferation.

A limitation of our study is that different methods were used to measure hemostatic factors at baseline and at follow-up. Activity assays were used at baseline, whereas antigen assays were used at follow-up. To determine the impact of this difference in assay methodology, we studied 40 healthy volunteers, matched for age and sex with the CARDIA participants. The correlation coefficients between activity and antigen assays were reasonably strong (0.81 for FVII, 0.61 for FVIII, and 0.95 for vWF). Thus, the increases in the hemostatic factors we observed during the 13-year interval were likely attributable to aging of the participants.

Further, although the assay methods used in our study at baseline and follow-up were different, their relationships to other measurements, such as IMT, were very similar (Figure 2). Another limitation is that the changes in clotting factors we observed could be a consequence, rather than the cause of the carotid thickening.

In conclusion, the main finding of this study is that FVII levels in the highest tertile are associated with an increase in CC-IMT. This association was observed when FVII was measured at 25 to 37 years of age as well as at 38 to 50 years of age, but lost statistical significance after multivariable adjustment for other CVD risk factors, most prominently BMI. Our observations suggest that persistently high levels of FVII, beginning as early as 25 to 37 years of age, are associated with the appearance of carotid thickening 13 years later.

Sources of Funding
This study was supported by grant HL-43758 and contracts NO1-HC-48049 and NO1-HC-95095 from the National Heart, Lung, and Blood Institute, and grant AG032136 from the National Institute on Aging, National Institutes of Health. The funders had no role in the design and conduct of the study, collection, management, analysis, and interpretation of the data, and preparation, review, or approval of the manuscript, except as required of all studies supported by NHLBI.

Disclosures
The authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. There are no conflicts of interest to disclose.

References
2. O’Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK. Carotid-artery intima and media thickness as a risk factor for myo-

Table 2. Associations of FVII and FVIII With Stroke

<table>
<thead>
<tr>
<th>Study</th>
<th>Age of Participants</th>
<th>Disease</th>
<th>FVII</th>
<th>FVIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPT†</td>
<td>40–64</td>
<td>Stroke and CHD</td>
<td>1.07</td>
<td>NR</td>
</tr>
<tr>
<td>ARIC</td>
<td>45–64</td>
<td>Stroke</td>
<td>1.03</td>
<td>1.93†</td>
</tr>
<tr>
<td>CRIC 11</td>
<td>&gt;65</td>
<td>Stroke</td>
<td>1.6</td>
<td>1.4†</td>
</tr>
</tbody>
</table>

*Hazard ratio; †relative risk; (95% CI).

TPT indicates Thrombosis Prevention Trial; ARIC, Atherosclerosis Risk in Communities; CHS, Cardiovascular Health Study; CHD, coronary heart disease; NR, not reported.


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Stroke. 2010;41:1417-1422; originally published online May 13, 2010; doi: 10.1161/STROKEAHA.110.580100
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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