High Factor VIII, von Willebrand Factor, and Fibrinogen Levels and Risk of Venous Thromboembolism in Blacks and Whites

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Venous thromboembolism (VTE) affects more than 300,000 people in the United States each year. However, it has been estimated that current diagnostic testing fails to identify pro-thrombotic risk in 50% of VTE patients. This article examines the relationship between levels of the pro-coagulant proteins factor VIII (FVIII), von Willebrand factor (VWF), and fibrinogen and risk of VTE in order to assess the impact of these novel risk factors. Data were collected from patients enrolled in the matched case-control Genetic Attributes and Thrombosis Epidemiology study. Crude and adjusted conditional logistic regression models were used to assess the impact of FVIII, VWF, and fibrinogen on risk of VTE. Before adjustment for independent predictors of VTE risk, high levels of FVIII, VWF, and fibrinogen were significantly associated with increased risk of VTE in both Blacks and Whites. After adjustment for ABO type, factor VII levels, hypertension, renal disease, recent surgery, diabetes, annual household income, alcohol use, and the other proteins of interest (FVIII , VWF, and/or fibrinogen), high FVIII and VWF levels were associated with increased risk of VTE in Blacks (OR: 1.97 [1.01–3.84] and 3.39 [1.58–7.27], respectively). High FVIII only was significantly associated with risk of VTE in Whites (OR: 2.35 [1.16–4.75]). Future research into the inclusion of these protein levels in risk models for VTE could help identify persons at highest risk. (*Ethn Dis.* 2014;24[2]:169–174)

Key Words: Venous Thromboembolism, Fibrinogen, Factor VIII, Von Willebrand Factor

Introduction

Venous thromboembolism (VTE) is estimated to affect 300,000–600,000 people in the United States each year;[1] it is the third leading cause of cardiovascular death,[2] and disproportionately affects Blacks.[3] Because current diagnostic testing for VTE fails to identify underlying pro-thrombotic tendency in about 50% of patients, identification of novel risk factors for VTE is essential.[4] Furthermore, several risk factors known to be associated with risk of VTE in Whites have been shown to have little impact on VTE risk in Blacks.[5–7] Identification of risk factors that may explain these racial differences could prove important in preventing VTE and reducing associated health disparities.

Several reports have indicated that high levels of pro-coagulant proteins may be independent risk factors for VTE.[8–12] Factor VIII (FVIII) circulates in plasma bound to von Willebrand factor (VWF) and is proteolytically cleaved during clot formation to yield activated FVIII which serves as a cofactor for the activation of Factor X (FX). Subsequently, activated FX serves as a cofactor for the conversion of prothrombin to thrombin, which acts on fibrinogen to form a fibrin clot. VWF stabilizes FVIII and provides an adhesive linkage between platelets and the subendothelium at sites of vascular injury. Elevated levels of FVIII have consistently been shown to be associated with risk of VTE,[8–10] while elevated levels of VWF and fibrinogen have not been consistently associated with an increased risk of VTE.[8,11–14] Furthermore, ethnic differences in mean steady-state levels of these proteins have been reported, with Blacks having higher average levels of both FVIII and VWF.[15,16] Factor VIIII, VWF, and fibrinogen, however, are acute phase reactants and are elevated in some conditions known to be risk factors for VTE. Our study examines the relationship between pro-coagulant protein levels measured after VTE events in a group of VTE cases compared to protein levels measured in a group of control patients and risk of VTE in both Blacks and Whites after adjustment for covariates.

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Materials and Methods

Study Population

The methods of the Genetic Attributes and Thrombosis Epidemiology (GATE) study have been described elsewhere.[17] Briefly, GATE is an age, sex, and race frequency-matched case-control study conducted in Atlanta, Georgia from January 1997 to September 2005 and designed to identify risk factors for VTE. Cases (n=1145) were selected from patients presenting with a first or recurrent VTE at either Crawford Long Hospital or Emory University Hospital and were confirmed by medical record review. Controls (n=1309) were selected from an Emory Healthcare primary care clinic. This report was limited to Black and White cases and controls who were not

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currently receiving anticoagulant therapy, who had available FVIII, VWF, and fibrinogen data, and who had a FVIII level above 50 IU/dL (n=1498). This project was approved by the Emory University and Centers for Disease Control and Prevention (CDC) Institutional Review Boards.

Laboratory Analyses

Blood samples were collected at the CDC laboratory (Atlanta, GA, USA). Samples were collected from controls upon recruitment and were collected from cases after completion of anticoagulant therapy. Samples were collected in siliconized evacuated glass tubes (Vacutainer, Becton Dickinsom and Company, Franklin Lakes, New Jersey, USA) containing .109M sodium citrate in a 1 to 9 volume ratio of citrate to blood. The tubes were centrifuged at 1,600 × g at 4 °C for 20 minutes followed by a repeat centrifugation of the separated plasma using the same protocol. The resulting platelet-poor plasma was stored in .5-mL aliquots at -70 °C until use.

Factor VIII, Factor VII (FVII), activated partial thromboplastin time (APTT), and fibrinogen were measured on the STA coagulation analyzer (Diagnostica Stago, Parsippany, New Jersey, USA). Factor VIII clotting activity was measured using a one-stage assay (Diagnostica Stago) that employs silica as an activator. Results of the assay were expressed as IU/dL by comparison with the international standard for FVIII and von Willebrand Factor (National Institute for Biological Standards and Control, Potters Bar, Herfordshire, UK). Factor VII clotting activity was measured using Factor VII-deficient plasma (Diagnostica Stago) and Neoplastin CI+ (Diagnostica Stago) and expressed as IU/dL by comparison with the international standard for FVII (National Institute for Biological Standards and Control). Activated partial thromboplastin time was measured via the STA-PTT A kit (Diagnostica Stago) using silica as an activator. Fibrinogen levels were determined using the STA-Fibrinogen kit (Diagnostica Stago) based on the clotting method outlined by Clauss.[18] The VWF antigen was measured by ELISA using polyclonal antiserum (Diagnostica Stago) and expressed as IU/dL by comparison with the international standard for FVIII and VWF (National Institute for Biological Standards and Control). The ABO serotype was determined using the reverse-typing method with A1 and B Referencells (Immucor, Norcross, Georgia, USA).

Anthropometric, Health Status, and Lifestyle Variables

Anthropometric variables (eg, sex, race, and age), health status variables (eg, hypertension diagnosis, recent surgery, and cancer diagnosis), and life style variables (eg, annual household income, alcohol consumption, and smoking status) were derived from responses to questions on a questionnaire administered by trained interviewers.

Statistical Analyses

All analyses were conducted using SAS Version 9.2 (SAS Institute, Cary, North Carolina, USA). Race specific abnormal levels of FVIII were defined as FVIII >200 IU/dL for Blacks and FVIII >150 IU/dl for Whites.[16] High VWF levels were defined as VWF >150 IU/dL. High levels of fibrinogen were defined as fibrinogen >4 g/L. Chi-square and Student t tests were used to assess the statistical significance of variations in the distribution of anthropometric, clinical, health status, and lifestyle characteristics by protein level. Because cases and controls were matched on age and sex, conditional logistic regression, conditioning on these variables, was used to assess the statistical significance of variations in the distribution of anthropometric, clinical, health status, and lifestyle characteristics by VTE case status. Variables such as BMI, FVII level, and APTT were assessed as continuous variables in these conditional logistic regression models. Adjusted odds ratios associated with high protein levels were estimated using conditional logistic regression, controlling for variables independently associated with risk of VTE as well as FVIII, VWF, and/or fibrinogen. The initial models included all variables that we judged as potential confounders. The final models were more parsimonious models that gave an effect estimate within 10% of the estimate from the full model and yielded the greatest average precision. These models included the following variables: FVIII, VWF, and/or fibrinogen as well as ABO type, FVII, hypertension diagnosis, hyperthyroid disease diagnosis, kidney disease diagnosis, recent surgery, diabetes diagnosis, income, and alcohol use. Statistical significance was assessed at the α=.05 level of significance.

RESULTS

Out of 1145 enrolled cases, 256 were eligible for this study (51 reported being a race other than Black or White and 889 did not return to the CDC laboratory for blood specimen collection after completion of anticoagulant therapy). Of the 256 eligible VTE cases, 152 cases were considered provoked, and 104 cases were considered idiopathic. The mean time between VTE event and return to CDC laboratory for testing was 10.5 (± 6.0) months. Out of the 1309 enrolled controls, 1242 were eligible for this study (45 reported being a race other than Black or White and 67 did not have a blood specimen). There were 667 White and 575 Black controls and 140 White and 116 Black cases eligible for the study. The mean FVIII, VWF, and fibrinogen levels for White controls were 142.3 IU/dL, 130.9 IU/dL, and 3.4 g/L, respectively. Whereas the mean FVIII, VWF, and fibrinogen levels for Black controls were 168.3 IU/dL, 149.0 IU/dL, and

Table 1. Significant independent predictors of VTE in the GATE Study

Covariate	Blacks				Whites			
	Cases *n*	Controls *n*	Odds Ratio[a]	*P*	Cases *n*	Controls *n*	Odds Ratio[a]	*P*
BMI, kg/m^2	116	575	.98	.47	140	667	1.07	<.01[b]
ABO Type								
O	43	43	1.00	-	44	286	1.00	-
A	30	158	1.14	.62	71	272	1.70	.01[b]
B	123	123	1.76	.02[b]	21	80	1.71	.07
AB	3	27	.70	.57	2	23	.60	.50
FVII (%)	116	575	1.00	.01[b]	140	667	1.00	<.01[b]
APTT (s)	116	575	.89	<.01[b]	140	667	.94	.05[b]
Hypertension								
Yes	51	268	.91	.68	65	192	2.10	<.01[b]
No	65	307			75	475		
Hyperthyroid Disease								
Yes	1	18	.26	.19	8	15	2.54	.04[b]
No	114	557			130	652		
Infection								
Yes	18	17	6.14	<.01[b]	28	40	4.08	<.01[b]
No	92	558			110	627		
Malignancy								
Yes	9	2	26.19	<.01[b]	12	4	16.22	<.01[b]
No	107	573			128	663		
Renal Disease								
Yes	15	8	9.80	<.01[b]	6	4	6.98	<.01[b]
No	101	566			134	662		
Surgery								
Yes	34	10	23.84	<.01[b]	70	17	36.28	<.01[b]
No	82	565			70	650		
Diabetes								
Yes	24	89	1.47	.15	21	41	2.52	<.01[b]
No	92	486			119	626		
Annual household income <$55,000								
Yes	82	371	1.46	.11	59	186	2.31	<.01[b]
No	30	202			76	478		
Education attainment <junior college								
Yes	75	343	1.22	.36	69	181	2.77	<.01[b]
No	41	232			71	486		
Alcohol consumption <1 drink/week								
Yes	106	438	3.48	<.01[b]	115	312	5.35	<.01[b]
No	10	137			25	355		
Physical Activity(% hours sitting/week)	116	575	1.00	.89	140	667	.99	<.01[b]

[a] Conditioned on age and sex.
[b] Significant at the α=.05 level of significance.

3.7 g/L, respectively. The mean FVIII, VWF, and fibrinogen levels for White cases were 179.7 IU/dL, 162.5 IU/dL, and 3.8 g/L, respectively. The mean FVIII, VWF, and fibrinogen levels for Black cases were 209.4 IU/dL, 195.8 IU/dL, and 3.9 g/L, respectively. There was no statistically-significant difference in the mean FVIII (183.6 IU/dL vs 177.5 IU/dL, *P*=.61), VWF (163.4 IU/dL vs 162.0 IU/dL, *P*=.92), or fibrinogen (3.79 g/L vs 3.78 IU/dl, *P*=.93) levels measured in White idiopathic VTE cases compared to White provoked VTE cases. Similarly, there was no statistically-significant different in the mean FVIII (213.4 IU/dL vs 205.8 IU/dL, *P*=.56), VWF (193.4 IU/dL vs 198.0 IU/dL, *P*=.78), or fibrinogen (3.79 g/L vs 3.97 g/L, *P*=.28) levels measured in

Table 2a. Crude and adjusted associations of protein levels with risk of VTE in Blacks

	Cases *n* (%)	Controls *n* (%)	Crude OR (CI)[a]	Adjusted OR (CI)[b]
High FVIII	60 (51.7)	148 (25.7)	3.03 (2.00–4.58)[c] *n*=691	1.97 (1.01–3.84)[c] *n*=559
High VWF	74 (77.1)	197 (41.7)	4.66 (2.78–7.78)[c] *n*=569	3.39 (1.58–7.27)[c] *n*=559
High fibrinogen	52 (44.8)	190 (33.0)	1.81 (1.18–2.78)[c] *n*=691	1.38 (.75–2.54) *n*=559
High FVIII and VWF	46 (47.9)	86 (18.2)	4.10 (2.55–6.59)[c] *n*=569	4.20 (2.24–7.89)[c] *n*=559
High FVIII and fibrinogen	26 (22.4)	70 (12.2)	2.15 (1.29–3.60)[c] *n*=691	1.12 (.52–2.38) *n*=559
High VWF and fibrinogen	35 (36.5)	77 (16.3)	3.12 (1.89–5.15)[c] *n*=569	1.63 (.84–3.14) *n*=559
High FVIII, VWF and fibrinogen	21 (21.9)	43 (9.1)	2.91 (1.61–5.27)[c] *n*=569	2.27 (1.11–4.63)[c] *n*=559

[a] Conditioned on age and sex.
[b] Conditioned on age and sex and controlling for FVIII, VWF, and/or fibrinogen, ABO type, FVII, hypertension diagnosis, hyperthyroid disease diagnosis, kidney disease diagnosis, recent surgery, diabetes diagnosis, income, and alcohol use.
[c] Significant at the α=.05 level of significance.

Black idiopathic cases compared to Black provoked cases.

Covariates that were found to be independent predictors of VTE are shown in Table 1. The strongest independent predictors of VTE risk included cancer diagnosis, renal disease diagnosis, and recent surgery. The variables shown in Table 1 were used in the full model to estimate adjusted odds ratios for risk of VTE.

Crude odds ratios for odds of VTE in patients with high protein levels compared to those with low protein levels are shown in Tables 2a (Blacks) and 2b (Whites). Before adjustment for independent predictors of VTE risk, high levels of FVIII, VWF, and fibrinogen were significantly associated with increased risk of VTE in both Blacks and Whites. Furthermore, combinations of high protein levels tended to be associated with greater increased risk of VTE, particularly for Whites. However, after adjustment for ABO type, FVII levels, hypertension, kidney disease, recent surgery, diabetes, annual household income, alcohol use, and other proteins of interest (FVIII, VWF, and/or fibrinogen), several associations were no longer significant. High FVIII and VWF were associated with increased risk of VTE in Blacks (OR: 1.97 [1.01–3.84] and 3.39 [1.58–7.27], respectively), with high levels of both proteins conferring even greater risk (OR: 4.20 [2.24–7.89]). High fibrinogen was no longer associated with significantly increased risk of VTE in Blacks (OR: 1.38 [.75–2.54]). High FVIII was the only protein level significantly associated with risk of VTE in Whites (OR: 2.35 [1.16–4.75]).

DISCUSSION

The objective of our study was to assess the association of FVIII, VWF, and fibrinogen levels and risk of VTE and to assess any differences in risk by race. Previous studies have implicated high FVIII, VWF, and fibrinogen levels as risk factors for VTE, with high FVIII levels being more consistently associated with increased risk.[8–12,14,19] However, many of these studies only adjusted for age and sex and failed to adjust for other covariates that impact both factor level and risk of VTE. We have used logistic regression models to control for such confounders and in doing so have eliminated high fibrinogen and VWF levels as risk factors for VTE in Whites. Both FVIII and VWF, however, remain independent predictors of risk for VTE in Blacks. While FVIII, VWF, and fibrinogen levels are related, controlling for levels of the proteins when assessing the effect of high levels of the other proteins allowed the assessment of the independent contribution of each protein to risk of VTE.

Table 2b. Crude and adjusted associations of protein levels with risk of VTE in Whites

	Cases *n* (%)	Controls *n* (%)	Crude OR (CI)[a]	Adjusted OR (CI)[b]
High FVIII	97 (69.3)	251 (37.6)	3.69 (2.48–5.48)[c] *n*=807	2.35 (1.16–4.75)[c] *n*=647
High VWF	64 (54.2)	140 (25.9)	3.37 (2.23–5.11)[c] *n*=540	1.23 (.58–2.59) *n*=647
High fibrinogen	49 (35.0)	103 (15.4)	2.85 (1.89–4.31)[c] *n*=807	1.44 (.71–2.89) *n*=647
High FVIII and VWF	54 (45.8)	114 (21.1)	3.12 (2.04–4.76)[c] *n*=658	1.56 (.78–3.09) *n*=647
High FVIII and fibrinogen	42 (30.0)	67 (10.0)	3.71 (2.37–5.80)[c] *n*=807	2.06 (.96–4.44) *n*=647
High VWF and fibrinogen	30 (25.4)	43 (8.0)	3.76 (2.23–6.35)[c] *n*=658	.87 (.38–1.99) *n*=647
High FVIII, VWF and fibrinogen	28 (23.7)	36 (6.8)	4.27 (2.46–7.40)[c] *n*=658	1.60 (.70–3.65) *n*=647

[a] Conditioned on age and sex.
[b] Conditioned on age and sex and controlling for FVIII, VWF, and/or fibrinogen, ABO type, FVII, hypertension diagnosis, hyperthyroid disease diagnosis, kidney disease diagnosis, recent surgery, diabetes diagnosis, income, and alcohol use.
[c] Significant at the α=.05 level of significance.

We have used logistic regression models to control for such confounders and in doing so have eliminated high fibrinogen and VWF levels as risk factors for VTE in Whites.

Recent publications have suggested the mechanism conferring VTE risk for high FVIII levels relates to constitutively high levels as opposed to spikes in levels, as is the case with the acute-phase response.[20,21] Our findings agree with these results. After controlling for variables likely implicated in acute-phase response (eg, diabetes diagnosis and recent surgery), we find high levels of FVIII remain an independent risk factor for VTE in both Blacks and Whites. Furthermore, after controlling for FVIII and VWF levels as well as other variables implicated in the acute-phase response, fibrinogen (a marker of the acute-phase response) is no longer associated with risk of VTE.

Our findings are the first to suggest that high VWF levels are associated with risk of VTE in Blacks only. Reports have suggested certain polymorphisms in the gene coding VWF are related to levels of VWF and that the distribution of these polymorphisms differs by race.[22] Perhaps these polymorphisms also confer increased risk for VTE and help explain the differing results between Blacks and Whites in our population; future studies are needed to assess this relationship. However, if these findings hold, high levels of VWF as a risk factor for VTE in Blacks may help explain some of the racial disparity in risk of VTE and could prove an important risk factor to assess in the clinical setting.

Because traditional diagnostic techniques for VTE fail to implicate an underlying inherited or acquired prothrombotic tendency in up to 50% of patients,[4] identification of novel risk factors such as FVIII, VWF, or fibrinogen levels could aid in the identification of patients at-risk for developing VTE before the event occurs. The findings in our study suggest measurement of FVIII activity could be considered when assessing VTE risk clinically in Whites and that both FVIII and VWF levels could be considered when assessing risk in Blacks. However, more work regarding the predictive value of these measurements is necessary before recommending the tests be used routinely in clinical settings.

The strengths of our study include its relatively large sample of VTE cases and controls and its ability to control for likely confounders of the association between FVIII, VWF, and fibrinogen levels and risk of VTE. A weakness of our study is the high rate of lost to follow-up for cases. A number of cases did not return to the CDC laboratory after completion of anticoagulant therapy for blood sample collection, possibly resulting in a biased case group. However, comparison of key indicators of health collected in the study questionnaire and completed by all cases indicated those who returned for blood sample collection were not significantly different than those cases who did return (Table 3). Another limitation of our study is the timing of procoagulant protein measurement. The VTE cases were identified prior to enrollment in the study, and procoagulant proteins were measured subsequent to enrollment. In order to prevent measurement

Table 3. Comparison of all cases enrolled in GATE to cases who were eligible for inclusion in analysis

	All Cases *N*=1145	Cases Analyzed *n*=256	*P*
Race, *n* (%)			
White	557 (50.9)	140 (54.7)	.31
Black	537 (49.1)	116 (45.3)	
Age, mean, years	48.7 years	49.9 years	.16
Sex, *n* (%)			
Female	576 (50.3)	126 (49.2)	.75
Male	569 (49.7)	130 (50.8)	
Diabetes, *n* (%)			
No	907 (79.2)	211 (82.4)	.25
Yes	238 (20.8)	45 (17.6)	
Hypertension, *n* (%)			
No	633 (55.3)	140 (54.7)	.85
Yes	511 (44.6)	116 (45.3)	
Alcohol consumption, *n* (%)			
>20 drinks/week	6 (.5)	1 (.4)	.61
8–20 drinks/week	26 (2.3)	7 (2.7)	
1–7 drinks/week	114 (10.0)	27 (10.6)	
<1 drinks/week	116 (10.1)	34 (13.3)	
Rarely/never	882 (77.1)	187 (73.1)	
Case type, *n* (%)			
Deep vein thrombosis only	691 (60.5)	166 (64.8)	.20
Pulmonary embolism only	232 (20.3)	53 (20.7)	
Deep vein thrombosis and pulmonary embolism	219 (19.2)	37 (14.5)	

bias, we required all cases to complete anticoagulant therapy before blood sample collection. However, it is possible levels of FVIII, VWF, or fibrinogen may differ before and after a biologically stressful event such as VTE. Our study assumed ample time between event and measurement allowed for the return to these proteins basal levels.

The results of our study indicate that high levels of FVIII and VWF are independent risk factors for VTE in Blacks and high levels of FVIII are a risk factor for VTE in Whites. After accounting for likely confounders, high fibrinogen levels were not a risk factor for VTE in this population. Future research into the inclusion of FVIII and VWF levels in risk models for VTE could help define those at highest risk for an event and could help explain some of the racial disparity in risk of VTE between Blacks and Whites.

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