A prospective study of venous thromboembolism in relation to factor V Leiden and related factors

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The aim of this study was to examine the occurrence of venous thromboembolism (VTE) in relation to factor V–related risk factors. Using a nested case-control design combining 2 population-based prospective studies, we measured factor V Leiden, HR2 haplotype, activated protein C (APC) resistance, and plasma factor V antigen in 335 participants who developed VTE during 8 years of follow-up and 688 controls. The overall odds ratio (OR) of VTE was 3.67 (95% CI, 2.20-6.12) in participants carrying factor V Leiden compared with noncarriers. APC resistance measured after predilution with factor V–deficient plasma conferred an OR of 2.58 (95% CI, 1.62-4.10). All 3 participants homozygous for the HR2 haplotype had a VTE, and the OR of VTE for homozygosity was estimated to be 5.5 (95% CI, 2.45-12.5). Carriers of the HR2 haplotype otherwise were not at increased risk of VTE overall (OR = 1.05; 95% CI, 0.64-1.72), but double heterozygotes for HR2 and factor V Leiden carried an OR of idiopathic VTE of 16.3 (95% CI, 1.7-159) compared with noncarriers. Factor V antigen also was not associated with VTE overall, but for participants with the combination of high factor V antigen plus factor V Leiden the OR of idiopathic VTE was 11.5 (95% CI, 4.2-31.4). In the general population, APC resistance and factor V Leiden were important VTE risk factors; homozygosity for the HR2 haplotype may be a risk factor but was rare; otherwise, HR2 haplotype and factor V antigen were not risk factors except in carriers of factor V Leiden. (Blood. 2002;99:2720-2725)

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Introduction

In the past decade, resistance to the natural anticoagulant activated protein C (APC) has been identified as a common and important cause of venous thromboembolism (VTE), that is, deep venous thrombosis (DVT) or pulmonary embolism (PE).[1] The most common cause of APC resistance is a mutation of factor V (Arg506Gln, factor V Leiden) at one of the cleavage sites for APC, present in approximately 5% of the US white population.[2] Population-based case-control studies suggest that heterozygosity for factor V Leiden carries a relative risk of incident VTE of approximately 3 to 8 and homozygosity carries a relative risk of 80.[3] Prospective data on factor V Leiden and incident VTE are available from only a nonpopulation-based study of US male physicians, in whom the relative risk of VTE was 2.7 for factor V Leiden heterozygotes.[2] Recent data have suggested factor V Leiden elevates risk for DVT more than for PE, suggesting a different pathologic character of clots associated with factor V Leiden.[4-10]

Little evidence to date suggests that a higher plasma level of factor V antigen is itself a risk factor for VTE.[11] However, recently an HR2 haplotype has been described, which includes an 4070A>G polymorphism in exon 13 of the factor V gene, replacing His (R1 allele) by Arg (R2 allele) at position 1299 of the B domain. The HR2 haplotype is reported to influence plasma factor V levels and to contribute to APC resistance.[12,13] Several case-control studies have disagreed on whether the HR2 haplotype increases risk of VTE, either by itself or in combination with factor V Leiden.[14-17] Additional population-based data on the risk related to the HR2 haplotype are needed, particularly prospective studies, in which interactions with plasma markers are free from the influence of treatment for VTE.

Because of the paucity of prospective population-based data on risk factors for VTE, we undertook the Longitudinal Investigation of Thromboembolism Etiology (LITE). This report concerns associations of VTE with factor V–related markers: factor V Leiden, HR2 haplotype, APC resistance, and plasma factor V antigen. In addition, we report the correlates of factor V antigen, about which there has been only one previous report.[5]

Materials and methods

Study population and baseline assessments

The LITE study is a prospective study of VTE occurrence in 2 pooled, multicenter, longitudinal population-based cohort studies: the Atherosclerosis Risk in Communities (ARIC) study and the Cardiovascular Health Study (CHS). The LITE study design, methods, and VTE incidence rates have been described in detail elsewhere.[18] In brief, 15 792 ARIC participants,

aged 45 to 64 years at baseline in 1987-1989, and 5201 CHS participants, aged 65 years or older at baseline in 1989-1990, were assessed for cardiovascular risk factors. An additional 687 African Americans were recruited to the CHS in 1992-1993. Blood was drawn from fasting participants in the morning in both studies, promptly centrifuged for 3 000g for 10 minutes, and the plasma was stored in $-70°$C freezers. Up to 3 follow-up examinations were performed every 3 years in the ARIC study and up to 9 follow-up examinations were performed annually in the CHS. Blood was stored from both the baseline examination and 3 years later in both studies. Baseline cardiovascular risk factors included in this paper were measured comparably in ARIC and CHS, as described elsewhere[18] and methods are not repeated here.

Nested case-control design

A nested case-control design was used to study prospective associations between VTE incidence and blood parameters measured in stored blood specimens. Potential cases of VTE were identified from baseline through September 1998. Hospital records were obtained and VTE events validated by 2 physicians as "definite DVT" (nearly always having a positive duplex ultrasound or a positive venogram), "probable DVT" (having a positive Doppler ultrasound or a positive impedance plethysmography), and "definite PE" (nearly always having ventilation-perfusion scans with multiple segmental or subsegmental mismatched defects or a positive pulmonary angiogram). Cases for this analysis included definite or probable DVT or definite PE. Cases were also classified as incident (no self-reported VTE history before baseline) or recurrent (self-reported VTE history before baseline) and idiopathic (no obvious cause) or secondary (associated with cancer, major trauma, surgery, marked immobility). From the ARIC study, 185 individuals with VTE were identified, 164 incident and 21 recurrent, 85 idiopathic and 100 secondary. Among 150 individuals with VTE events in the CHS, 120 were incident and 30 recurrent, 68 were idiopathic, and 82 secondary. Of the 335 events, 237 had venous thrombosis only, 52 had a PE only, and 46 had both. Of those with venous thrombosis only, 220 involved the veins of the legs, pelvis, or the inferior vena cava.

Controls were selected at random from the ARIC and CHS cohorts being followed. To facilitate selection, potential controls were first assigned follow-up times at random between 0 days and the maximum number of follow-up days that subjects could have participated in the study. Controls then were selected at a ratio of 2.1 per case, frequency matched to the cases by age (5-year groupings), sex, race (African American, white), follow-up time (cases' event date within 2 years of controls' assigned date) and study (ARIC, CHS). This control selection process ensured that the set of potential controls included a random selection of individuals who could have been diagnosed with VTE (had it occurred) at the assigned follow-up time.[19] Selection yielded 390 controls for the 185 incident cases in the ARIC study and 298 for the 150 incident cases in the CHS.

Laboratory methods

After selection of cases and controls, stored samples of DNA and plasma were retrieved from $-70°$C storage freezers. If baseline plasma samples were limited, previously thawed, or exhausted for a participant, a sample was retrieved from the plasma repository for the next visit (approximately 3 years after baseline); if neither sample was available it was considered missing. The percentages of ARIC subjects having plasma from baseline, the year 3 visit, or missing were, respectively, 65%, 25%, and 10%. The respective percentages for CHS plasma were 80%, 14%, and 6%. DNA was missing or permission to use it was not given for 8% of ARIC participants and 11% of CHS participants. For the factor V–related variables of interest in the present analysis, the percentage of missing samples did not significantly differ between cases and controls, between incident versus recurrent cases, between idiopathic versus secondary cases, or between DVT versus PE cases.

We detected the presence or absence of the factor V Leiden (1691G>A, Arg506Gln) mutation using standard methods.[20] We identified the HR2 haplotype of the factor V gene by screening for the presence or absence of the R2 polymorphism, a 4070A>G transition in exon 13 of the factor V gene.[14] A 703-bp fragment was amplified by polymerase chain reaction

(PCR) and digested with the restriction enzyme *Rsa*I, as previously described.[12,13] Presence of the R2 allele is identified by digestion of the 703-bp fragment to fragments of 492 and 211-bp in size.

Plasma for assessment of APC resistance had been centrifuged at 4°C in ARIC and at room temperature in CHS. Pilot studies suggested processing temperature should not affect results. The APC ratio was measured in subjects not taking warfarin using the IL Test APC Resistance Kit (Instrumentation Laboratory, Milan, Italy), based on the activated partial thromboplastin time (aPTT) assay in the absence and presence of human APC. The APC ratio is lower in individuals with APC resistance than in individuals with normal response to APC. We used 2 methods, a first-generation APC sensitivity ratio (APC-SR)[21] and a newer assay incorporating predilution with factor V–deficient plasma to improve detection of factor V–dependent APC (modified APC-SR).[22] Measurement was done on an ACL Futura Coagulation System (Instrumentation Laboratory). Laboratory interassay and intra-assay variation is about 7%. We calculated the abnormal cutoff value, based on the manufacturer's instructions, as a function of the APC ratio for control participants who did not carry factor V Leiden (n = 542). For all batches, measured values for high and low plasma controls fell on the appropriate side of the APC-SR cutoff value.

Factor V antigen was measured using a sandwich-type enzyme-linked immunosorbent assay, which used polyclonal antibodies for capture and a monoclonal antibody directed against the amino terminal–derived heavy chain of factor V for signal (antibodies from Haematologic Technologies, Essex Junction, VT). Factor V antigen was measured in citrated plasma processed at 4°C and not previously thawed since storage. During thawing, exposure of the sample to 37°C conditions was avoided. Three coefficients of variation were calculated for 3 control pools (mean factor V concentration shown) used during the study: 4.9% (mean 6.64 μg/mL), 5.8% (mean 8.64 μg/mL), and 6.7% (mean 8.51 μg/mL).

Statistical analyses

Study variables were described univariately by calculating prevalences (%) or mean values. Relations among pairs of independent variables were described by cross-tabulation or Spearman correlations. APC resistance (yes, no) and the APC ratio (continuous variable) were both examined. Unconditional logistic regression was used to calculate odds ratios (ORs) and 95% CIs of VTE in relation to risk factors. A number of lifestyle, physiologic, and hemostatic factors have already been evaluated for association with VTE in the LITE cohort.[18] Adjustment was made for factors previously associated with VTE, including age (continuous) in all models and race, sex, body mass index (BMI; continuous), and factor VIII (continuous) in additional models. Logistic regression analyses were repeated, stratified by study (ARIC and CHS) and by race (white and African American). However, there were insufficient events to obtain stable estimates for African Americans alone. Logistic regression analyses for subgroups of cases (incident versus recurrent VTE, idiopathic versus secondary VTE, and DVT versus PE) were performed using all controls as the comparison group. Interactions of factor V Leiden with age, APC-SR, HR2, and factor V antigen, hypothesized a priori, were examined for idiopathic VTE by cross-classification.

Analyses using plasma variables were performed first excluding, then including, the 4% (n = 12) of VTE events that occurred before the visit in which samples of blood were available. Because results were similar, these cases were retained in the final analysis.

Results

Descriptive information and frequencies of factor V Leiden and HR2 haplotype

The overall samples of patients and controls at baseline were 45 years and older; 53% were women, and 76% were white.

Approximately 14% of cases and 4% of controls carried factor V Leiden, but cases and controls had similar frequencies of the

Table 1. Frequencies (n) of factor V Leiden and HR2 haplotype in VTE cases and controls, LITE

	V Leiden				HR2 haplotype			
	Genotyped	−/−	−/+	+/+	Genotyped	−/−	−/+	+/+
Cases	301	260	38	3	302	276	23	3
White	229	190	36	3	232	207	22	3
African American	72	70	2	0	70	69	1	0
Controls	630	604	26	0	620	569	51	0
White	494	468	26	0	486	439	47	0
African American	136	136	0	0	134	130	4	0

HR2 haplotype (Table 1). Few African Americans carried factor V Leiden or the HR2 haplotype. Among white controls, 5% were heterozygous for factor V Leiden and 10% were heterozygous for HR2. Overall, only 5 participants (0.6% of 914) were heterozygous carriers of both mutations.

Prevalence of APC resistance and its relation with genotypes and other factors

The prevalence of APC resistance was 18.6% in cases and 12.9% in controls by the first-generation assay (APC-SR ≤ 2.57) and 14.6% in cases and 6.2% in controls by the modified assay (modified APC-SR ≤ 2.12). The prevalence of APC resistance by APC-SR was 79% among carriers of factor V Leiden and 10% among noncarriers. Using the modified APC-SR these prevalences were 98% and 2%, respectively, indicating near perfect sensitivity and specificity for factor V Leiden for modified APC-SR. The HR2 haplotype was not significantly ($P > .05$) associated with APC resistance.

APC-SR (continuous variable) was correlated positively at $P < .05$ with aPTT (r = 0.19) and was correlated negatively with protein C (r = −0.21), factor VII (r = −0.13), von Willebrand factor (r = −0.14), and factor VIII (r = −0.07). The mean APC-SR was higher ($P < .05$) in men (3.46) than women (3.24), in African Americans (3.44) than whites (3.31), and in current smokers (3.46) than never smokers (3.27), but did not differ by diabetes or hormone replacement status.

Factor V antigen levels and relation with genotypes and other factors

The mean ± SEM factor V antigen level was 17 ± 4.6 μg/mL in homozygous factor V Leiden carriers (n = 3), 16 ± 0.9 μg/mL in heterozygous carriers, and 13 ± 0.2 μg/mL in noncarriers ($P = .03$

for difference). There was no relation of factor V antigen levels with HR2 genotype ($P = .28$).

Factor V antigen was correlated positively at $P < .05$ with BMI (r = 0.22), factor VIII (r = 0.19), von Willebrand factor (r = 0.17), fibrinogen (r = 0.18), and low-density lipoprotein (LDL) cholesterol levels (r = 0.12), and was correlated negatively with high-density lipoprotein (HDL) cholesterol (r = −0.14) and alcohol intake (r = −0.12). The mean factor V level was higher ($P < .005$) in men (14.2 μg/mL) than women (13.0 μg/mL), and in female nonusers of hormonal replacement (13.4 μg/mL) than users (10.5 μg/mL), but did not differ by race or smoking or diabetes status.

Associations with VTE

All 3 participants who were homozygous for factor V Leiden had a VTE, and their OR using the Hardy-Weinberg equilibrium assumption was estimated[23] to be 25 (95% CI, 10-66). Subsequently, homozygotes were pooled with the heterozygotes to compute the population-wide risk of carrying factor V Leiden. The occurrence of VTE, adjusted for age, was 3.67-fold higher in carriers of factor V Leiden than in noncarriers (Table 2). This OR was 3.69 in whites, 4.05 in ARIC, and 3.16 in CHS. The OR for factor V Leiden was about twice as high in participants with a recurrent VTE (OR = 5.79) than those with incident VTE (OR = 3.31 overall; 3.93 in ARIC and 2.36 in CHS). The OR was nearly 3 times as high in idiopathic VTE (OR = 5.91 overall; 6.16 in ARIC and 3.95 in CHS) versus secondary VTE (OR = 2.02). The OR for factor V Leiden was somewhat higher in those with DVT only (OR = 3.76) than in those with PE only (OR = 2.86), but CIs overlapped. When adjusted for race, sex, BMI, and factor VIII level, as well as age, the overall OR was 3.56 (95% CI, 2.11-6.03), suggesting little confounding by these variables.

All 3 participants homozygous for HR2 had a VTE, and their OR was estimated[23] to be 5.5 (95% CI, 2.45-12.47). However,

Table 2. Age-adjusted OR and 95% CI for VTE in relation to factor V–related variables, LITE

Group (no. of VTE)	Factor V Leiden*		HR2 Haplotype*		APC resistant by APC-SR		APC resistant by modified APC-SR		Factor V antigen quintile 5 versus 1	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Overall (n = 290-302)	3.67†	2.20-6.12	1.05	0.64-1.72	1.54†	1.05-2.25	2.58†	1.62-4.10	1.60†	1.03-2.48
Whites only (n = 217-232)	3.69†	2.18-6.24	1.13	0.68-1.89	1.44	0.93-2.24	2.51†	1.54-4.09	1.35	0.81-2.23
ARIC (n = 158-170)	4.05†	2.15-7.64	1.08	0.55-2.12	1.53	0.93-2.52	2.56†	1.45-4.49	1.41	0.79-2.51
CHS (n = 132-138)	3.16†	1.31-7.60	0.98	0.47-2.04	1.54	0.86-2.78	2.65†	1.16-6.09	1.92	0.91-4.04
Incident (n = 253-257)	3.31†	1.93-5.67	1.05	0.62-1.77	1.33	0.88-2.00	2.28†	1.39-3.74	1.40	0.88-2.25
Recurrent (n = 37-45)	5.79†	2.52-13.33	1.07	0.37-3.10	3.33†	1.60-6.93	4.86†	2.13-11.08	3.05†	1.19-7.81
Idiopathic (n = 129-138)	5.91†	3.34-10.46	1.18	0.62-2.24	1.85†	1.14-2.99	4.11†	2.40-7.02	1.97†	1.09-3.56
Secondary (n = 161-166)	2.02†	1.01-4.02	0.95	0.50-1.79	1.30	0.80-2.12	1.52	0.81-2.85	1.34	0.77-2.31
DVT (n = 208-216)	3.76†	2.17-6.53	1.02	0.58-1.78	1.60†	1.05-2.44	2.63†	1.58-4.37	1.52	0.94-2.48
PE (n = 44-47)	2.86†	1.04-7.85	0.79	0.24-2.62	1.03	0.42-2.53	2.21	0.88-5.59	1.24	0.48-3.19
Both (n = 38-42)	4.21†	1.62-10.93	1.59	0.59-4.23	1.84	0.81-4.17	2.73†	1.07-6.96	3.68	0.96-14.03

*Homozygous plus heterozygous.
†$P < .05$.

Table 3. Adjusted OR (95% CI) for VTE in relation to percentile of APC-SR and factor V antigen, LITE

	Percentiles						
	Below 5	5 to 20	20 to 40	40 to 60	60 to 80	80 to 95	Above 95
APC-SR*							
OR, age-adjusted	1.66	1.46	1.22	1.15	1.08	1.0‡	
(95% CI)	(0.84-3.29)	(0.90-2.37)	(0.77-1.92)	(0.72-1.82)	(0.68-1.71)	(reference)	
OR, multivariate adj†	1.49	1.12	1.06	1.02	0.97	1.0‡	
(95% CI)	(0.74-3.02)	(0.67-1.88)	(0.66-1.71)	(0.63-1.66)	(0.60-1.55)	(reference)	
Modified APC-SR*							
OR, age-adjusted	4.50	1.36	1.49	1.15	1.01	1.0‡	
(95% CI)	(2.22-9.10)	(0.82-2.25)	(0.93-2.36)	(0.72-1.83)	(0.63-1.63)	(reference)	
OR, multivariate adj†	4.45	1.46	1.57	1.21	1.03	1.0‡	
(95% CI)	(2.12-9.31)	(0.85-2.52)	(0.95-2.59)	(0.74-2.00)	(0.61-1.73)	(reference)	
Factor V antigen							
OR, age-adjusted	1.0§		1.15	1.12	1.19	1.47	2.04
(95% CI)	(reference)		(0.74-1.80)	(0.72-1.75)	(0.76-1.85)	(0.92-2.37)	(1.03-4.04)
OR, multivariate adj†	1.0§		1.19	1.03	1.01	1.17	1.84
(95% CI)	(reference)		(0.74-1.90)	(0.63-1.63)	(0.63-1.63)	(0.70-1.95)	(0.90-3.74)

*A higher APC-SR indicates greater sensitivity to APC.
†Adjusted for age, race, sex, BMI, and factor VIII.
‡Reference for ORs is highest quintile.
§Reference for ORs is lowest quintile.

when heterozygous and homozygous carriers of HR2 were pooled, HR2 was not statistically significantly associated overall, or in any subgroups, with VTE occurrence (Table 2). Additional adjustment for race, sex, BMI, and factor VIII had no impact on the HR2 OR.

The patterns of association for APC resistance (Table 2), as expected, generally paralleled those for factor V Leiden. The age-adjusted ORs for APC resistance were larger for modified APC-SR (OR = 2.58, overall) than for APC-SR (OR = 1.54). Furthermore, considered together, APC resistance by modified APC-SR was associated independently with VTE, but APC resistance by standard APC-SR was not.

The association of VTE with percentiles of continuous APC-SR is shown in Table 3. Although most of the excess risk of VTE was related to an APC ratio in the "resistant" range, individuals with values in the lower end of the normal range (below quintile 2) also had somewhat elevated VTE risk. Additional adjustment for race, sex, BMI, and factor VIII attenuated the ORs for groupings of continuous APC-SR, but not for modified APC-SR (Table 3). After excluding participants with factor V Leiden, there was no association of continuous APC-SR with venous thrombosis (data not shown).

Factor V antigen levels in the highest quintile were associated ($P < .05$) with increased VTE occurrence adjusted for age (OR = 1.60 overall and even greater in recurrent and idiopathic subgroups; Table 2). The OR was particularly elevated for factor V antigen above the 95th percentile versus the first quintile (OR = 2.04), but multivariate adjustment attenuated the OR (OR = 1.84; Table 3). Six values for factor V antigen were high (> 132 µg/mL) in ARIC participants. When they were excluded, the overall age-adjusted VTE OR was reduced to 1.47 (95% CI, 0.94-2.29) for the highest versus lowest quintile and 1.61 (95% CI, 0.81-3.23) for the highest 5% versus the lowest quintile.

Interactions with factor V Leiden

The ORs of idiopathic VTE in relation to factor V Leiden and other factors jointly are shown in Table 4. Although both greater age and factor V Leiden increased the odds of VTE occurrence, there was no striking elevation of VTE odds in participants with both risk factors. This was also true for the combination of factor V Leiden and APC resistance. However, both HR2 and elevated factor V

antigen were synergistic (ie, relative risks supra-additive[24]) with factor V Leiden. Although few people had these joint risk factors, the OR was 16.3 for double heterozygosity of factor V Leiden and HR2, compared to people with neither mutation, and was 11.5 for factor V Leiden plus a high factor V antigen level.

Discussion

Descriptive findings

The frequency of factor V Leiden heterozygosity among population-based white controls in the LITE study (4%) was generally consistent with other studies,[1,2,25] as was the virtual absence of factor V Leiden in

Table 4. Age-adjusted OR and 95% CI for idiopathic VTE in relation jointly to factor V Leiden and other factors, LITE

Variable	Factor V Leiden*	No. of events	OR	95% CI
Age				
Younger than 65 y	No	58	1.00	Reference
65 y and older	No	52	1.69	0.84-3.41
Younger than 65 y	Yes	20	7.25	3.57-14.76
65 y and older	Yes	8	7.91	2.44-25.65
APC resistant by APC-SR				
No	No	85	1.00	Reference
Yes	No	8	0.83	0.38-1.81
No	Yes	5	5.30	1.49-18.81
Yes	Yes	18	5.70	2.85-11.42
HR2*				
No	No	98	1.00	Reference
Yes	No	10	1.11	0.54-2.25
No	Yes	24	5.29	2.90-9.64
Yes	Yes	3	16.33	1.68-159
Factor V ag†				
Low	No	79	1.00	Reference
High	No	21	1.12	0.71-2.09
Low	Yes	13	5.05	2.29-11.15
High	Yes	13	11.49	4.21-31.35

Ag indicates antigen.
*Heterozygous or homozygous.
†Low factor V is in the lower 4 quintiles; high is in the upper quintile.

African American controls.[25-27] The frequency of heterozygosity for the HR2 haplotype among whites also was similar to other studies,[15-17] but HR2 heterozygosity was infrequent among African Americans. In a separate LITE study report, we observed a higher risk of VTE among African Americans compared to whites,[18] and given race differences in risk factors such as factor V Leiden, additional study of African American populations is needed.

The modified APC-SR assay, which incorporated predilution with factor V–deficient plasma, proved to be highly sensitive and specific for factor V Leiden, as has been previously reported.[28-31] The standard APC-SR was less sensitive and specific for factor V Leiden. Regardless of method, APC resistance was not significantly influenced by HR2 haplotype. Previous studies on whether HR2 affects APC resistance have been inconsistent.[5-17]

The standard APC-SR (continuous variable) was found to be correlated with several factors (aPTT, factor VIII, race, sex, smoking, and HDL cholesterol) as reported by others.[32-34] Increased factor VIII, in particular, has been an increasingly recognized contributor to APC resistance. African Americans have higher factor VIII levels than whites,[35] but paradoxically less APC resistance, presumably because factor V Leiden is rare among African Americans. APC-SR was shown here to be negatively correlated with von Willebrand factor, factor VII, and protein C. The protein C association with APC-SR is particularly noteworthy because it suggests that lower APC sensitivity may cause a compensatory rise in the protein C concentration.

There are few previous data on determinants of factor V antigen levels. Kamphuisen et al reported factor V antigen to be correlated positively with smoking and factor VIII.[11] We corroborated the factor VIII correlation, but found no relation with smoking. We also observed higher levels of factor V antigen in men than women and in factor V Leiden carriers than noncarriers. This suggests a hypothesis that either APC resistance itself, or factors such as increased thrombin generation with subsequent platelet activation as a result of APC resistance,[36] might increase the expression or release of factor V from platelets. There also were positive associations of factor V with von Willebrand factor, fibrinogen, and LDL cholesterol, and negative associations with alcohol intake and HDL cholesterol.

Associations with VTE

The OR of VTE in relation to factor V Leiden was similar in the LITE study to previous studies,[3] and as might be expected for a genetic risk factor was greater in idiopathic than secondary events. Some investigators have reported that factor V Leiden is a stronger risk factor for DVT than PE.[4-10] However, we found little evidence for this. We also did not corroborate a stronger association of factor V Leiden with VTE in older than in younger participants.[37] Whether factor V Leiden is associated with recurrent events is somewhat controversial.[38] We found factor V Leiden associated strongly with recurrent events. Recurrence here was defined from self-reported history at baseline; reanalysis based on evidence of prior VTE in the medical record gave similar results. Although the associations of factor V Leiden with incident and recurrent VTE are now becoming clear, ongoing clinical trials are required to define any role for long-term anticoagulation among heterozygotes with thrombosis.[39]

As would be expected from its higher sensitivity and specificity for factor V Leiden, APC resistance measured by the modified APC-SR assay was a better marker of VTE risk than measured by the standard APC-SR assay. However, APC resistance by standard APC-SR did not predict idiopathic VTE after stratification for factor V Leiden. Thus, in contrast to the findings from the Leiden

Thrombophilia Study, which reported that APC resistance was associated with VTE in the absence of factor V Leiden,[40] our data do not support a role for measurement of APC-SR in addition to factor V Leiden. Reasons for this difference from the Leiden study are uncertain but could relate to our study having a smaller number of cases, an older and ethnically diverse population, or longer storage of plasma samples.

Occurrence of VTE has not been related consistently to the HR2 haplotype.[14-17] We also found no increased risk for HR2 heterozygous carriers but HR2 homozygotes were estimated to be at approximately 5-fold increased risk of VTE. There also appeared to be synergy of HR2 with factor V Leiden for double heterozygotes, as was previously suggested.[14] However, both the presence of homozygosity for HR2 or double heterozygosity for factor V Leiden and HR2 were very rare, so any clinical application of HR2 testing is questionable.

Elevated factor V antigen was associated positively with VTE incidence, although not statistically significantly after excluding 6 high values ($> 132 \mu g/mL$) or after adjusting for other risk factors. We are uncertain what accounted for the high factor V values; those participants' values for BMI, lipids, fibrinogen, factor VIII, and von Willebrand factor were unremarkable. A high factor V also proved to be synergistic with factor V Leiden, with the joint relative risk being 11.5. The Leiden Thrombophilia Study also reported no overall association of VTE factor V antigen and possible synergism with factor V Leiden.[11]

Study limitations

A strength of the LITE prospective study is that plasma samples for incident VTE cases, the large majority of all cases, were collected before the onset of VTE. The observed associations between VTE and nongenetic markers therefore should be less susceptible to bias than the associations reported by case-control studies. The LITE plasma samples were stored up to 12 years, which could have introduced random error and weakened associations. However, stability of numerous coagulation factors during long-term storage under similar conditions was recently demonstrated.[41] Follow-up of cohort participants was high and VTE events were classified by standardized criteria. However, whether we ascertained all clinically recognized VTE depended on participants' accurate reporting of hospitalizations and on their physicians' diagnostic work-up of suspected VTE events. Of course, clinically unrecognized VTE were missed, but should have been rare enough among controls to introduce little bias.

Conclusion

In this general population study, APC resistance and factor V Leiden were important VTE risk factors; homozygosity for the HR2 haplotype may be a risk factor but was rare; otherwise, HR2 haplotype and factor V antigen were not risk factors except in carriers of factor V Leiden. Any role of clinical testing for factor V–related factors among patients with thrombosis remains to be fully defined, although the findings here provide important new data for consideration of this question.

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