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Associations of the β -Fibrinogen Hae III and Factor XIII Val34Leu Gene Variants with Venous Thrombosis

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Abstract

Introduction—The factor XIII Val34Leu (100 G→T) and β -fibrinogen Hae III (-455 G→A) gene variants have been associated with reduced risk of venous thrombosis, but not in all studies.

Methods—We investigated the associations of these polymorphisms with risk of venous thrombosis in a prospective, population-based study of 21,680 men and women aged 45–100 years at enrollment. Factor XIII 100 G/T and β -fibrinogen -455 G/A were analyzed on stored DNA from 511 thrombosis cases and 1028 control subjects without thrombosis during follow up.

Results—The β -fibrinogen A allele was present in 24.4% of cases and 32.3% of controls. Compared to GG subjects, the age, race, and sex adjusted odds ratio (OR) of venous thrombosis was 0.77 (95% CI 0.59–0.99) for GA subjects, and 0.60 (95% CI 0.31–1.16) for AA subjects. The adjusted OR of thrombosis associated with factor XIII 100 G/T was 1.01 (95% CI 0.81–1.26) for GT subjects and 0.45 (95% CI 0.44–1.19) for TT subjects, compared to GG. For both genotypes, ORs of thrombosis were similar in whites and non-whites, although there were no non-white fibrinogen AA cases. β -Fibrinogen -455GA or AA attenuated the thrombosis risk associated with obesity (from 2.14 to 1.25) and factor V Leiden (from 3.89 to 2.36).

Conclusions— β -fibrinogen -455 G/A, but not factor XIII 100 G/T, was associated with a lower risk of venous thrombosis in this general population sample. β -Fibrinogen -455A may attenuate the increased thrombosis risk associated with obesity or factor V Leiden.

Keywords

venous thrombosis; risk factor; fibrinogen; factor XIII; factor V Leiden; obesity

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Venous thrombosis (VT) occurs in 1–2 of every 1000 adults yearly. Despite its high incidence, the etiology of VT is incompletely understood. Risk factors for VT include obesity, cancer, immobilization, surgery, trauma and pregnancy, and inherited tendencies such as factor V Leiden, prothrombin 20210A and deficiencies of anticoagulant factors, protein C, protein S and antithrombin.

A common polymorphism in Factor XIII, a G100T substitution in exon 2 of the α -subunit resulting in a valine to leucine substitution at position 34 (FXIII Val34Leu), appears to lower the risk of VT, although not all studies agree [1–6]. A meta-analysis of 12 studies suggested a small inverse association [7]. Factor XIII (FXIII) is a tetrameric ($\alpha_2\beta_2$), pro-transglutaminase that is activated by thrombin and stabilizes fibrin clots by forming fibrin γ -chain dimers [3,8,9]. It also cross-links α_2 -plasmin inhibitor to the fibrin polymers [10]. The Val34Leu amino acid substitution is located 3 amino acids from the thrombin cleavage site. It appears to increase activation of FXIII, and lead to a higher dissociation index, but does not seem to affect cross-linking [5,9]. Premature activation of FXIII depletes the pool of available FXIII and results in fibrin clots that are looser and more susceptible to fibrinolysis [8]. In one study the association of Val34Leu with VT was most apparent in participants with higher fibrinogen [11], corroborating in vitro studies that suggested the fibrin clots of the Leu variant are more permeable or loose in the setting of higher fibrinogen [12].

Studies have disagreed on whether a β -fibrinogen polymorphism, Hae III or -455 G/A, is inversely associated with VT risk [13–17]. Elevated fibrinogen levels may increase the risk of VT, though this has not been observed consistently [13,18,19]. The fibrinogen -455 G/A polymorphism is located on the β chain in the promoter region and has been associated with higher fibrinogen levels [14,20,21]. This would suggest that the β -fibrinogen variant is related to a hypercoagulable state and thrombosis; however in one large study the A allele was associated unexpectedly with lower VT risk than the G allele [13]. In a study among African Americans, the A allele was less common than in whites and was not associated with VT [15].

We evaluated the associations of the FXIII Val34Leu and β -fibrinogen -455 G/A polymorphisms, as well as fibrinogen levels, with deep vein thrombosis and pulmonary embolus in the Longitudinal Investigation of Thromboembolism Etiology (LITE), a population-based study of the incidence and risk factors for VT. In addition to studying main effects and associations in whites and blacks separately, we determined whether these polymorphisms were associated with reduced VT risk when other common risk factors were present, specifically Factor V Leiden and obesity.

Materials and Methods

Subjects

The LITE is a prospective study that combined two U.S. cohorts, the Cardiovascular Health Study (CHS) and the Atherosclerosis Risk in Communities (ARIC) study [22]. Both population-based studies examined risk factors at baseline and subsequent development of cardiovascular diseases in six communities. Details of CHS and ARIC have been published previously [23,24]. In 1987–89, 15,792 men and women between the ages of 45–64 were enrolled in ARIC, with 27% African Americans. In 1989–90, 5,201 men and women age 65 and older were enrolled in CHS, with 3% African American. Another 687 African American participants were enrolled in 1992–93. Extensive information was collected from participants at baseline, including medical history, self-reported ethnicity, height and weight. All participants provided written informed consent with methods approved by local institutional review committees.

A total of 548 cases of VT were validated using standardized criteria from baseline to 2002 as previously described [22]. Each study maintained contact with all participants and identified all hospitalizations. Potential cases of hospitalized VT were identified and hospital records were abstracted for validation of cases by two physicians. VT events required a positive duplex or Doppler ultrasound or venogram, impedance plethysmography or a high probability ventilation-perfusion scan or chest computed tomography. Cases were ascertained in two phases, first from enrollment to the end of 1998, then from that time to the end of 2002. Approximately 43% of the cases were idiopathic. Among secondary events cancer was the most common precipitant, present in 191 of 314 cases.

A nested case-control design was used to assess genotypic risk factors for VT in LITE. Controls were randomly chosen from the ARIC and CHS cohorts at a ratio of approximately 2 controls per case, yielding a total of 1097 controls. Cases and controls were frequency matched according to age, gender, race, follow-up time and study (ARIC/CHS).

Laboratory Methods

In ARIC and CHS, fasting morning blood samples were obtained at baseline. Citrated blood was centrifuged at 4°C, and frozen at -70°C until analysis in central laboratories. Several plasma and DNA measures were performed in the nested case-control sample, including Factor V Leiden [25]. Among nested case control study subjects, DNA was missing or consent to use DNA was not given in 48 of 1645 (2.9%) participants. Genetic assays were done in two batches corresponding to the two case ascertainment phases. In the first phase assays were done using PCR with restriction digestion. In the second phase, analyses were done in multiplex using the MassARRAY homogenous MassEXTEND assay of the Sequenom system (San Diego, CA). The FXIII Val34Leu variant was detected using PCR and MseI restriction digestion in the first phase [2] and by Sequenom forward and reverse primers 5'-ACGTTGGATGGGTATGCTCATAC-CTTGACG-3' and 5'-ACGTTGGATGTAATGCAGCGGAAGATGACC-3' in the second phase. The extension primer was 5'-CAGTGGAGCTTCAGGGC-3'. Fibrinogen -455G/A was determined by PCR, and HaeIII restriction enzyme digestion in the first phase, and by Sequenom forward and reverse primers 5'-ACGTTGGATGCACATTATGATATAACATTAC-3' and 5'-ACGTTGGATGGCTTATGTTTTCTGACAATG-3' in the second phase. The extension primer was 5'-TTCTATTTCAAAGGGGC-3'. In ARIC, fibrinogen was measured in the entire cohort during enrollment by the Clauss method with reagents and calibration materials (Fibriquik) from General Diagnostics (Organon-Technika Co) [26]. In CHS, fibrinogen was measured in the entire cohort during enrollment by a modification of the Clauss method using a BBL Fibrometer (Becton Dickinson) [27]. Because of unavailable DNA or unsuccessful DNA analysis there were 63 participants missing results for the β -fibrinogen genotype, 62 for factor XIII and 66 for factor V Leiden. This left 511 cases and 1028 controls for analysis.

Statistical Analysis

Version 8 of SAS was used for statistical analysis. We determined the baseline characteristics for cases and controls, and compared prevalences of the polymorphisms in cases and controls and in ethnic groups using Chi-squared tests. The relative risk of VT by baseline fibrinogen level was estimated using Cox proportional hazards regression adjusting for age, sex and race. Logistic regression adjusting for age, sex and race was used to determine the odds ratio (OR) and 95% confidence interval (CI) of developing VT in the presence of each polymorphism. Analyses were repeated in subgroups defined by VT case type (incident or recurrent; idiopathic or secondary); parent study membership; Factor V Leiden status (heterozygote plus homozygote or wild type); ethnic group (white or non-white); and body mass index (above or below 30 kg/m²).

The associations for the factor XIII gene polymorphism were tested comparing 100 GT and TT to GG separately. For the subgroup analyses defined above, participants with GT and TT were combined. The fibrinogen variant was analyzed in similar fashion, first comparing -455 GA or AA to GG separately, and then combining GA and AA for subgroup analyses.

Results

Table 1 shows that cases and controls were similar for the matching factors and fibrinogen levels. There was a higher prevalence of obesity and Factor V Leiden among cases. Fibrinogen levels did not differ significantly by β -fibrinogen genotype in cases or controls. Results were similar stratifying by race (data not shown). Of the 511 cases, 452 reported no VT at baseline and 220 were idiopathic. There were 319 cases and 639 controls among ARIC participants and 192 cases and 389 controls among CHS participants. Nearly all nonwhites in the analysis were black.

There was no association of baseline age, sex and race-adjusted fibrinogen level with risk of VT; the hazard ratios for quartile 2, 3 and 4 compared to quartile 1 were 0.99, 1.12 and 0.95, respectively (hazard ratio per standard deviation higher fibrinogen 1.00).

Table 2 shows the distributions of the β -fibrinogen and FXIII genotypes by case/control status and ethnicity. Both the β -fibrinogen -455 AA and the FXIII 100 TT genotypes were present in less than 10% of cases and controls. Heterozygote individuals were more abundant, comprising about 1/4 of cases and controls for β -fibrinogen and about 1/3 of cases and controls for FXIII. The β -fibrinogen A allele was more common in whites than non-whites, present in 38.6% of white controls and 12.8% of non-whites ($p < 0.0001$). In cases but not controls, the FXIII T allele was more common in whites than non-whites (45.1% of white cases; 34.3% of non-white cases; $p = 0.01$). β -fibrinogen AA and factor XIII TT were more prevalent among controls than cases.

Table 3 shows the ORs of VT by genotype, adjusted for age, sex and race. The β -fibrinogen A allele was associated with a reduced risk of VT. Compared to those with fibrinogen -455GG, subjects with fibrinogen -455 GA had a 23% lower risk of VT, while AA subjects had a 40% lower risk; the latter did not reach statistical significance. Results were similar for idiopathic VT. The FXIII T allele was not clearly associated with reduced risk of VT. Compared with the GG genotype, FXIII GT was associated with a nonsignificant 1% higher risk of VT while the TT genotype was associated with a nonsignificant 55% lower risk. Associations were less apparent for idiopathic VT, with an 18% lower risk with the TT genotype. Analyses limited to incident VT yielded similar results for both genotypes, with ORs of 0.81 and 0.67 for fibrinogen -455 GA or AA, and 0.97 and 0.73 for factor XIII GT or TT, respectively. Recurrent events were too few for reliable analysis ($n=49$).

To study whether associations might differ by age groups we performed secondary analyses stratified by study membership (ARIC or CHS). For β -fibrinogen -455 GA and AA the ORs were 0.66 (95% CI 0.47–0.92) and 0.61 (95% CI 0.26–1.38) in ARIC participants, while these were 0.96 (95% CI 0.64–1.42) and 0.56 (95% CI 0.18–1.76) in CHS participants. For Factor XIII GT and TT the ORs were 0.78 (95% CI 0.59–1.05) and 0.56 (95% CI 0.30–1.20) in ARIC and 1.51 (95% CI 1.05–2.17) and 1.21 (95% CI 0.53–2.80) in CHS. The associations of β -fibrinogen genotype with VT were similar comparing whites and non-whites (not shown), while the factor XIII genotype was not associated with VT among whites (ORs 1.02 and 0.99 respectively for GT and TT). Among non-whites (137 cases and 273 controls), factor XIII GT was not associated with VT (OR 0.98), while the TT genotype was associated with a 90% lower risk (OR 0.10; 95% CI 0.01–0.79).

Table 4 shows the joint associations of the β -fibrinogen genotype and obesity or factor V Leiden with VT. Compared to those without obesity and without a β -fibrinogen -455 A allele, subjects with obesity in the absence of an A allele were 2-fold more likely to develop VT. However, obese subjects with an A allele were not at increased risk of VT (OR 1.25; 95% CI 0.83–1.89). The 95% CI for these odds ratios overlapped. Similar analyses assessing the combined association of β -fibrinogen -455A and factor V Leiden with VT showed that among those with factor V Leiden, a β -fibrinogen -455A allele slightly attenuated the risk of VT. The ORs of VT for factor V Leiden in the absence or presence of an A allele were 3.89 and 2.36, respectively. Presence of one or more T alleles for the FXIII variant did not modify the risk of VT for either obesity or factor V Leiden (data not shown).

Discussion

In this study, the β -fibrinogen -455A allele was associated with a lower risk of VT compared to the G allele, with heterozygotes (GA) having a 23% lower risk and homozygotes (AA) a 40% lower risk than homozygous GG. Fibrinogen -455A attenuated the risk of VT associated with obesity and slightly reduced the risk associated with factor V Leiden. The FXIII Val34Leu variant was not as clearly associated with a lower risk of VT, contrary to our hypothesis and some previous reports.

The inverse association of β -fibrinogen -455GA with VT risk is perplexing, as the A allele has been associated with higher fibrinogen levels, which would suggest an increased risk of thrombosis. Similar to our findings, the Leiden Thrombophilia Study reported an inverse association of -455A and VT and no association of this genotype and fibrinogen level. In contrast to our study, they reported that elevated fibrinogen was associated with increased risk of VT [13]. van Hylckama Vlieg et al further characterized these data, reporting that fibrinogen above the median was associated with increased risk of VT, but only among those over age 45 [19]. They concluded that this may be due to cumulative age-related risk factors. In the LITE study consisting only of those above age 45, elevated fibrinogen, even at extreme levels, was not a VT risk factor in our previous report [18] and in updated data here.

Given the unclear associations of fibrinogen level with VT and the findings here and elsewhere on the -455G/A polymorphism [13], it appears likely that this gene variant is linked to another causal gene variant, and is not etiologically related to VT. It is also possible that this variant influences functional aspects of fibrinogen not assessed by measuring its concentration. Further study is needed regarding the relationship of β -fibrinogen -455G/A, fibrinogen function and other fibrinogen gene cluster variants. In the LITE study adjustment for β -fibrinogen -455G/A did not change the association of α -fibrinogen Thr312Ala with VT risk (Rasmussen-Torvik, in press).

Our results demonstrated that the association of obesity with VT is attenuated by the presence of a β -fibrinogen -455A allele, reducing the OR of VT from 2.0 to that of the non-obese population. Similarly, -455A lowered the risk associated with factor V Leiden from 3.9-fold to 2.4-fold. More precise estimates of gene-obesity and gene-gene interactions in VT risk are needed using larger sample sizes.

We confirmed that the β -fibrinogen variant is less common in nonwhites than whites [15]. Studies of European whites demonstrated prevalences of the AA genotype in control groups ranging from 6.3% to 10% [13,16], which compares to 4.6% here. Despite prevalence differences, the relationship of -455GA to VT did not differ by ethnicity in our study.

Although other studies reported that the FXIII T allele was inversely associated with VT [1, 2,5,6,28], we did not confirm this with statistical certainty. Only homozygosity was associated with a nonsignificantly lower risk, which was less prominent among those with idiopathic VT,

but more apparent in participants below age 65 at baseline (OR 0.56). Catto reported an inverse association for the Val34Leu mutation in a retrospective study of white Northern Europeans [1]. Franco reported an inverse association of TT with VT in 189 cases and 187 controls (predominantly whites) [2]. In that study, only 3 cases and 18 controls had the FXIII TT genotype (compared to our study with 24 cases and 65 controls). Salazar-Sanchez reported an inverse association of TT with VT among Latin American residents of Costa Rica, with only 4 cases and 13 controls being TT [6].

In the Leiden Thrombophilia Study, the largest case-control study of this topic, there was a non-significant, weak inverse association of the T allele with VT in Dutch patients. In contrast, Balogh demonstrated no association of the Val34Leu mutation with VT in a large Hungarian population under age 45 [3], and Margaglione showed no association in a larger referred population [4]. The meta-analysis by Wells and colleagues reported data from 12 retrospective case-control studies including 3165 cases of VT, primarily under age 70 [7]. They reported an odds ratio of 0.63 (95% CI 0.46–0.86) for TT and 0.89 (95% CI 0.80–0.99) for GT subjects. Our study of 511 cases is larger than any study included in that meta-analysis, so results should be reconsidered.

There are a few possible reasons for differences in findings between our study and others regarding FXIII Val34Leu. First, if the gene variant is associated with surviving an event, then it might be more common in cases than controls in a retrospective study, possibly explaining an inverse association. Second, unlike many studies, LITE included secondary VT and this might result in underestimation of risk. However, subgroup analysis suggested a weaker association for idiopathic than secondary VT. This is counter to expectations for most causal genetic thrombophilias of larger associations for idiopathic events. Third, differences among studies in prevalences of other common unknown genetic disorders might influence findings for less penetrant disorders.

Strengths of our study include its prospective design, diverse ethnicity and large sample size. We also assessed interactions of these variants with other common VT risk factors. Limitations include limited power for subgroup analyses by age group, race and event type, and these must be interpreted cautiously. For example there were 138 cases among nonwhites and too few recurrent events for analysis.

In summary, we report a reduced risk of VT with the β -fibrinogen A allele but not the FXIII T allele. The β -fibrinogen variant attenuated the increased risk of VT with concomitant obesity and perhaps factor V Leiden. Both the FXIII and the β -fibrinogen variants were less common in non-white ethnic groups, but associations with VT did not differ. Given differing results in various studies, further study of the β -fibrinogen variant is warranted, including studies addressing its interaction with obesity and other risk factors.

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Abbreviations

VT
venous thrombosis

OR

odds ratio

LITE

Longitudinal Investigation of Thromboembolism Etiology

CHS

Cardiovascular Health Study

ARIC

Atherosclerosis Risk in Communities

CI

confidence interval

FXIII

factor XIII

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Table 1
Baseline characteristics of VT cases and controls, LITE

Risk Factor	Cases (n=511)	Controls (n=1028)
Age (years) *		
45–50	64 (13%)	142 (14%)
51–60	160 (31%)	327 (32%)
61–70	175 (34%)	323 (31%)
71+	112 (22%)	236 (23%)
Male Gender *	284 (56%)	573 (56%)
Ethnicity *		
White	373 (73%)	754 (73%)
Non-white	138 (27%)	274 (27%)
Body Mass Index $\geq 30 \text{ kg/m}^2$	193 (38%)	254 (25%)
Fibrinogen (mg/dL)	(n=504)	(n=1017)
β -fibrinogen -455 GG	305 [†]	313 [‡]
β -fibrinogen -455 GA	308	315
β -fibrinogen -455 AA	305	309
Factor V Leiden	(n=505)	(n=1026)
Heterozygous	48 (9%)	33 (3%)
Homozygous	4 (1%)	0

* Matching factor

[†] p=0.66 for level by genotype in cases

[‡] p=0.79 for level by genotype in controls

Table 2

Genotype frequency of VT cases and controls by ethnicity, LITE

	Number genotyped	β-Fibrinogen GG	GA	AA	Number genotyped	Factor XIII GG	GT	TT
Cases	507	375 (73.4)	120 (23.7)	12 (2.4)	510	295 (57.8)	191 (37.4)	24 (4.7)
Nonwhite	137	126 (92.0)	11 (8.0)	0	137	90 (65.7)	46 (33.6)	1 (0.7)
White	370	249 (67.3)	109 (29.5)	12 (3.2)	373	205 (55.0)	145 (38.9)	23 (6.2)
Controls	1027	701 (68.3)	289 (28.1)	37 (3.6)	1025	585 (57.1)	375 (36.6)	65 (6.3)
Nonwhite	273	238 (87.1)	33 (12.1)	2 (0.7)	273	168 (61.5)	87 (31.9)	18 (6.6)
White	754	463 (61.4)	256 (34.0)	35 (4.6)	752	417 (55.4)	288 (38.3)	47 (6.2)

Values shown are frequency (%)

Table 3

Odds ratio of overall and idiopathic VT by genotype, LITE

	Overall VT				Idiopathic VT			
	Number of Cases	Number of Controls	OR*	95% CI	Number of Cases	Number of Controls	OR*	95% CI
<u>β-fibrinogen</u>								
GG	375	701	1.0	Reference	162	701	1.0	Reference
GA	120	289	0.77	0.59–0.99	53	289	0.80	0.56–1.13
AA	12	37	0.60	0.31–1.16	5	37	0.59	0.23–1.53
<u>Factor XIII</u>								
GG	295	585	1.0	Reference	122	585	1.0	Reference
GT	191	375	1.01	0.81–1.26	86	375	1.1	0.82–1.51
TT	24	65	0.45	0.44–1.19	11	65	0.82	0.42–1.59

* ORs adjusted for age, sex and race.

Table 4
Odds Ratio of VT with β -fibrinogen -455G/A and obesity or factor V Leiden, LITE

Obesity	β -Fibrinogen GA or AA	Number of Cases	Number of Controls	OR *	95% CI
No	No	222	520	1.0	Reference
No	Yes	91	249	0.83	0.62–1.11
Yes	No	152	177	2.14	1.62–2.82
Yes	Yes	41	77	1.25	0.83–1.89
Factor V Leiden	β -Fibrinogen GA or AA	Number of Cases	Number of Controls	OR *	95% CI
No	No	337	681	1.0	Reference
No	Yes	114	331	0.75	0.58–0.97
Yes	No	36	19	3.89	2.19–6.92
Yes	Yes	16	14	2.36	1.13–4.91

* OR adjusted for age, sex and race