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The FXIII Val34Leu polymorphism in venous and arterial thromboembolism

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ABSTRACT

Background and Objectives. **Several hereditary disorders affecting coagulation factors have been identified as prothrombotic risk factors. Recently, the common Val34Leu polymorphism of the A-chain factor XIII gene, associated with high factor XIII activity, has been identified as a protective genetic factor against occlusive arterial and venous diseases in British and Finnish populations. The aim of our study was to investigate the role of this polymorphism in arterial and venous thromboembolic disorders in a distinct population.**

Design and Methods. **We analyzed the prevalence of this polymorphism in three case/control studies of consecutive patients from the south of Spain diagnosed as having acute coronary syndromes (101), acute cerebrovascular events (104), and deep venous thrombosis (97).**

Results. **No significant differences were detected in the prevalence of genotypes or alleles between patients and controls.**

Interpretation and Conclusions. **The Leu 34 allele does not play an important role in the development of thromboembolic episodes in the Spanish population.**
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Key words: Factor XIII, venous thrombosis, arterial thrombosis, polymorphisms, risk factors

During the last decade it has been demonstrated that mutations in genes that encode proteins involved in thrombus formation play an important role in the predisposition to a persistent hypercoagulable state.[1] Accordingly, several hereditary disorders, particularly those affecting the physiologic anticoagulant (antithrombin III, proteins C and S) and procoagulant systems (factor V Leiden and prothrombin 20210 A/G), have been well established as risk factors for venous thromboembolism.[2-5] In contrast, there is not yet clear evidence concerning the potential role of these mutations in arterial thrombosis.[4,5] The search for thrombotic risk factors has also involved elements of the fibrinolytic system with contradictory results.[6] However, it is possible that other common polymorphisms affecting coagulation or fibrinolytic factors could play a significant role in thrombotic disorders.

Factor XIII (FXIII) has a pivotal role in the coagulation process. By means of its transglutaminase activity, FXIII cross-links fibrin monomers and thus, clots have enhanced mechanical strength and become more resistant to fibrinolysis.[7] Recently, one common polymorphism affecting the A-chain of the FXIII (Val34Leu) has been associated with thrombotic disorders. Surprisingly, the less frequent allele (Leu34) does not increase the risk of thrombosis, but has been described as being a protective factor against myocardial infarction[8,9] and venous thrombosis.[10,11]

The aim of the present study was to investigate this FXIII polymorphism in relation to the development of thromboembolic disorders in a Mediterranean population. Thus, we conducted three case/control studies of patients diagnosed as having acute coronary syndromes, acute cerebrovascular events and venous thrombosis. Furthermore, we analyzed the Val34Leu polymorphism in 456 subjects from the same geographical area in south-eastern Spain who had no history of vascular disease.

Design and Methods

Selection of patients and controls

The present study was approved by the local Ethics Committee, and all participants gave their informed consent to be studied. Genotypic analyses were performed on 101 patients who survived an acute coronary event, admitted to the Coronary Unit with an established diagnosis of coronary heart disease (CHD) according to *World Health Organization* criteria.[12] We also enrolled 104 consecutive patients with cerebrovascular disease (CVD) referred to our institution. Diagnosis was attained according to the classification of cerebrovascular diseases of the *National Institute of Neurological Disorders and Stroke ad hoc Committee*.[13] Moreover, 97 consecutive patients with a confirmed diagnosis (by compression ultrasonography or contrast venography) of deep venous thrombosis (DVT) were evaluated. All cases of CHD, CVD or DVT were age, sex and race matched to a control, who had no history of vascular disease. Controls were selected by reviewing patient charts from a population of patients admitted to the hospital who had no history of vascular disease. CVD and CHD controls were additionally chosen in order to match selected risk factors for arterial thromboembolic disease

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Table 1. Age and sex of subjects studied, and prevalence of selected risk factors for arterial thrombosis among patients and controls.

	CHD			CVD			DVT			
	Patients (n = 101)	*Controls (n = 101)*	*p*	*Patients (n = 104)*	*Controls (n = 104)*	*p*	*Patients (n = 97)*	*Controls (n = 97)*	*p*	*General population (n=456)*
Age (yr): range	34-85	34-86	0.53	24-88	24-88	0.93	19-87	19-87	0.98	18-90
X±SD	62.9±11.1	62.9±11.1		65.8±13.8	65.6±13.8		60.9±14.9	60.8±14.8		48.0±20.0
Male sex (%)	73.3	73.3	1	51.9	51.9	1	56.7	56.7	1	48.9
Hypertension (%)°	49.5	38.6	0.12	46.2	38.5	0.26	–	–	–	–
Current/former smoker (%)	51.5	50.5	0.89	29.8	39.4	0.15	–	–	–	–
Hypercholesterolemia (%)	42.6	31.7	0.11	31.7	28.9	0.65	–	–	–	–
Type I or II diabetes (%)	37.6	29.7	0.23	35.6	32.7	0.66	–	–	–	–

**Student's t-test (for age) and the chi-square test (for all other variables) were used to compare the values for case patients and controls. °Hypertension was defined as a systolic blood pressure ≥ 140 mm Hg at the time of admission to the hospital. Hypercholesterolemia was defined as a total serum cholesterol level ≥ 5.72 mmol/L (2.2 g/L) at the time of admission to the hospital. Abbreviations: CHD, coronary heart disease; CVD, cerebrovascular disease; DVT, deep venous thrombosis.*

(smoking history, blood pressure, cholesterol level and diabetes status) with his or her respective patient (Table 1). Pre-specified subgroup analyses were done with stratification by age (≤60, >60), sex, and type of coronary and cerebrovascular acute event.

Furthermore, to determine the frequency of the studied polymorphism in our area, genetic analyses were also performed on 456 non-related and unselected subjects who had no documented history of vascular disease. Because the prevalence of the FXIII V/L 34 polymorphism could vary among different populations, we limited the study to the Mediterranean Caucasian population.

Blood collection and DNA isolation.

Blood samples were obtained by atraumatic venipuncture collection into 1:10 volume of EDTA (Vacutainer, Becton Dickinson, Meylon, France). Total genomic DNA was obtained from peripheral blood after lysis with SDS and proteinase K treatment of buffy coat. DNA was purified using phenol/chloroform and ethanol precipitation.[14]

DNA studies

Genomic polymerase chain reaction (PCR) of the FXIII exon 2 gene was performed using the following primers: 5' GACCTTGTAAAGTCAAAAATGTC3' and the mutagenic 5' TGGTGCCCCGGGGCGTCAACCTGCAAG3' (corresponding to nucleotides 67-89, and 215-238, respectively) [nucleotide number according to Grundmann *et al*.[15]]. The mutated nucleotide in the reverse primer allowed the identification of the 214 G/T (Val34Leu) polymorphism of the FXIII gene by restriction of the PCR product [148 base pairs (bp)] with *Bsa* HI (New England Biolabs, Beverly, MA, USA), followed by electrophoresis in acrylamide gels stained with $AgNO_3$.[16] The G allele (Val34) displayed a band pattern of 119 bp, whereas the presence of a 134 bp band was distinctive of the T allele (Leu34) (Figure 1).

Statistical analysis

Student's t-test was used to compare age; all other variables were analyzed by the chi-square test. A *p* value ≤ 0.05 was considered to indicate statistical significance. The strength of the association of the polymorphism with the occurrence of thrombosis was estimated by calculation of the odds ratio (OR) with the EpiInfo software and the Cornfield method for the calculation of 95% confidence intervals (CI).

Results

Characteristics of the study population

Table 1 shows age and sex of the study subjects. No significant differences were found in the prevalence of

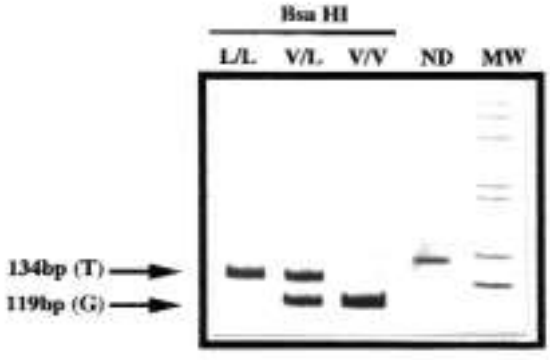

Figure 1. Representative genotypes of the Val34Leu polymorphism of the A-chain FXIII gene. MW: molecular weight marker (1 Kb ladder GIBCO-BRL), bp: base pairs, ND: not digested.

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Table 2. Genotype and allele frequencies of the Val34Leu polymorphism of the A-chain FXIII gene in three case/control studies and in the general populations from different countries.

	CHD		CVD		DVT		General population					
	Patients % n=101	Controls % n=101	Patients % n=104	Controls % n=104	Patients % n=97	Controls % n=97	Spain % n=456	UK[17] % n=436	UK[19] % n=113	Brasil[11] % n=187	France[18] % n=244	Finland[9] % n=344
Val/Val	67.3	67.3	57.7	68.3	62.9	68.0	65.1	58.3	65.5	58.8	52.9	56.7
Val/Leu	31.7	30.7	40.4	27.9	35.1	28.9	32.0	36.0	33.6	31.6	43.4	37.2
Leu/Leu	1.0	2.0	1.9	3.8	2.1	3.1	2.9	5.7	0.9	9.6	3.7	6.1
p	0.840		0.137		0.613							
Val	0.83	0.83	0.78	0.82	0.80	0.83	0.81	0.76	0.82	0.75	0.75	0.75
Leu	0.17	0.17	0.22	0.18	0.20	0.17	0.19	0.24	0.18	0.25	0.25	0.25
p	0.895		0.270		0.601							

selected risk factors for arterial thrombosis between patients and controls in the CHD and CVD case/control studies.

Genotyping of the Val34Leu polymorphism of the A-chain FXIII gene

Factor XIII genotype and allele frequencies of all groups included in our study are shown in Table 2.

Factor XIII Val34Leu polymorphism was analyzed by SSCP, allele-specific PCR amplification or solid-phase minisequencing.[9,17,18] We have developed a simple and reproducible PCR-ASRA method that allows rapid determination of the Val34Leu genotype by the use of a mutagenic primer. The frequency of the Leu 34 allele in the general population from our region was slightly lower than that previously reported in other Caucasian countries (Table 2).[8-11,17-19] We did not detect age or sex-dependent differences in the frequency of these alleles and the distribution of genotypes was not significantly different from Hardy-Weinberg proportions.

Finally, the case/control studies did not reveal statistical differences in the genotype or allele frequencies between patients and controls ($p > 0.05$). No differences related to age, sex, presence of any other risk factor, or type of coronary or cerebrovascular acute event were detected in relationship to the Val34Leu genotype (data not shown).

Discussion

Factor XIII is present in plasma as a heterotetramer, with two proenzyme units (A chain) and two carrier proteins (B chain). Factor XIII is activated by cleavage of thrombin at the Arg37-Gly38 bond of the A-chain, and a conformational change induced by Ca^{++}.[20,21] While the other enzymes in the clotting cascade are serine proteases, FXIII has transglutaminase activity, which plays an essential role cross-linking fibrin in the final stages of blood coagulation, increasing resistance against premature fibrinolysis.[7] The clinical relevance of FXIII in hemostasis is demonstrated by the severe bleeding tendency of patients with congenital FXIII deficiency.[22]

There is a wide range of plasma FXIII transglutaminase activity in the normal population that is not directly dependent on FXIII levels.[19] Recently, distinct genetic polymorphisms of the A-chain FXIII gene have been correlated with FXIII specific activity,[19] and one of them, Val34Leu has attracted all attention for two reasons. First, this amino acid change is located close to the thrombin cleavage site and could, therefore, affect the activation process or the activity of the resulting enzyme. Second, this polymorphism has been associated with arterial or venous vascular disorders.[8-11] However, some conflicting points derive from the latter observation. First, this would be the first common polymorphism involved in both, arterial and venous thrombosis. So far, all genetic risk factors for venous thrombosis have a minor influence on arterial thrombosis, probably conditioned by the distinct features of arterial and venous circulation.[23] Second, the less frequent Leu 34 allele has been defined as being the first protective genetic factor against vascular thrombosis, as a result of its lower prevalence in patients than in controls. However, in a biallelic system, this affirmation necessarily implies that the other allele (Val 34) should be more prevalent among patients. According with previously presented data,[8–11] there is a significant thrombotic risk associated with the presence of the Val 34 allele (OR 1.70 [95% CI 1.20-2.42] and OR 1.5 [95% CI 1.18-1.88], for myocardial infarction and deep venous thrombosis, respectively). Therefore, the protective or risk role of the Val34Leu polymorphism of FXIII in vascular disorders should be clarified. Finally, several recent studies demonstrate a significantly higher FXIII specific transglutaminase activity in carriers of the Leu 34 allele.[19,24,25] These data, together with the risk of coronary artery disease associated with high FXIII activity[26] make it difficult to explain the suggested protective role of Leu 34 allele in thromboembolic disease.

To assess the association between the Val34Leu

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polymorphism of the A-chain FXIII gene and vascular diseases, we compared the allele prevalence of this polymorphism among patients with distinct diseases (DVT, CVD and CHD) and control subjects. Since thromboembolic disease is a multifactorial disorder, genetic and environmental factors act additively or synergistically to determine the risk of an individual of developing thrombosis. Therefore, our strategy for identification of polymorphisms predisposing people to suffer from the disease, or protecting from having it, was to avoid overrepresentation of classic vascular risk factors in the CVD and CHD groups compared to controls, matching patients and controls for race, age, sex, hypertension, hypercholesterolemia, smoking, and diabetes. The frequencies of the FXIII genotypes or alleles were similar between arterial or venous thromboembolic patients and their respective control groups. Consequently, the findings of the present study suggest that this polymorphism of FXIII is unlikely to be associated with arterial or venous thromboembolic events. However, considering these case/control studies, several points should be borne in mind when interpreting our results. First, the study was performed in thromboembolic event survivors. Therefore, a survival bias can not be avoided in the disease-association study, and likely early mortality could lead to an underestimation of the alleles associated with higher or lower FXIII activity, as has been recently suggested.[9] Second, our study was performed in Caucasian subjects from the Mediterranean area. Since the prevalence of this polymorphism could vary geographically, and it is linked to other FXIII polymorphisms also associated with the activity of FXIII,[19] the FXIII Val34Leu polymorphism may have a different predictive value for thromboembolic disease in diverse populations. Therefore, these data can not exclude the possibility of an association between the Val34Leu FXIII polymorphism and thrombotic risk in other populations, and consequently, the relevance of FXIII polymorphisms should be investigated further.

Contributions and Acknowledgments

JC designed the study, performed the DNA analyses in the DVT case/control study and wrote the manuscript. RGC carried out the CHD case/control study. JAI analyzed the CVD case/control study. JR and CM performed the analysis of this polymorphism in the general population group. VV was involved in the design of the study, interpretation of its data and critically reviewed the manuscript. The criteria for the order in which the names of the authors appear are based on their contribution to the design, execution of the study, analysis and interpretation of data.

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Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

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Potential implications for clinical practice

- The factor XIII Val34Leu polymorphism is unlikely to be associated with arterial or venous thromboembolic events in Spanish population. Therefore, screening for this polymorphism would not help to identify subjects at higher risk of developing thromboembolic events.

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