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Inherited Thrombophilia is Associated With Deep Vein Thrombosis in a Colombian Population

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The development of venous thromboembolism is influenced by a variety of genetic and environmental risk factors. A few studies have ascertained whether thrombophilic defects are risk factors for venous thromboembolism in Latin American populations with a variable degree of admixture, such as the Colombian population. To address this issue, we conducted a case–control study involving 100 consecutive patients with deep vein thrombosis and 114 healthy controls from the Hospital Universitario San Vicente de Paúl, Medellín, Colombia. Activated protein C resistance (APC resistance) was detected in 25/99 patients vs. 6/114 controls (OR = 6.08, 95% CI = 2.23–17.47). Ten of 100 patients carried the factor V Leiden mutation vs. 1/114 controls (OR = 12.56, 95% CI = 1.61–267). APC resistance was associated with the factor V Leiden mutation in only 10/25 patients. The prothrombin G20210A mutation was found in 4/100 patients, but none of the controls ($P < 0.05$). There was no significant difference in the proportion of homozygous carriers of methylenetetrahydrofolate reductase C677T variant among patients and controls. In conclusion, in our studied population, factor V Leiden, APC resistance, and prothrombin G20210A were associated with an increased risk of deep vein thrombosis. However, the frequencies of these thrombophilic defects and of APC resistance associated with factor V Leiden was lower than the corresponding frequencies previously reported for Caucasian populations. Further study is required to assess the influence of ethnicity on thrombophilia. Am. J. Hematol. 81:933–937, 2006. © 2006 Wiley-Liss, Inc.

Key words: factor V Leiden; prothrombin G20210A; activated protein C resistance; methylenetetrahydrofolate reductase; thrombophilia; deep vein thrombosis

INTRODUCTION

Venous thromboembolism (VTE) is the third most prevalent cardiovascular disorder after ischemic heart disease and stroke, and it can lead to pulmonary embolism, a life-threatening event. Moreover, acute thrombotic events may be followed by significant morbidity, including post-thrombotic syndrome and recurrent VTE. One in 1,000 individuals is afflicted by VTE annually [1]. VTE is a multifactorial disorder that results from the interaction of genetic and acquired factors. In the last decade, there has been a significant advance in the understanding of inherited thrombotic disorders. In the past, up to 70% of VTE patients without identifiable risk factors (i.e., malignancy, surgery, trauma, and immobilization) but with clinical features that suggested inherited thrombosis were termed "idiopathic." Now, molecular diagnosis and current labo-

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TABLE I. Frequency of Distribution of the Studied Polymorphisms in the Group of Patients With DVT Versus Control Group

Polymorphism	Genotype	Patients ($n = 100$)	Controls ($n = 114$)	OR
Factor V 1691	GG	90 (90)[a]	113 (99.1)	0.08 [0–0.62][b]
	GA	10 (10)	1 (0.9)	12.56 [1.61–267]
	AA	0	0	N/A
Prothrombin 20210	GG	96 (96)	114 (100)	0 [0–1.33]
	GA	3 (3)	0	N/A
	AA	1 (1)	0	N/A
MTHFR 677	CC	27 (27)	36 (31.6)	0.8 [0.42–1.51]
	CT	49 (49)	56 (49.1)	1 [0.56–1.76]
	TT	24 (24)	22 (19.3)	1.3 [0.65–2.67]

N/A = not applicable.
[a]Values in parentheses indicate percentages.
[b]Values in parentheses indicate 95% confidence intervals.

ratory techniques allow accurate determination of the genetic basis of inherited thrombophilia in at least half of the Caucasian patients [2].

The prevalence of inherited thrombophilic disorders varies in different human populations. The genetic predisposition to VTE is influenced by the ethnicity of the population, which varies from country to country in the American continents, and is related to social and political characteristics of the settlement. The Colombian population can be defined as a ''tri-ethnic population,'' whose genetic background is roughly 70% Caucasian (mainly Spanish), 15% Amerindian, and 15% African [3,4]. This is a different distribution from that reported for Mexico, Argentina, and the United States [2,5,6]. In this study, we evaluated Colombian patients with deep vein thrombosis (DVT) and healthy blood donors, in order to assess the frequency of primary thrombophilic defects, including activated protein C resistance (APC resistance), the factor V Leiden mutation (factor V G1691A), the prothrombin G20210A mutation, and the methylenetetrahydrofolate reductase thermolabile variant (MTHFR C677T), as risk factors for thrombosis in this population.

PATIENTS AND METHODS

Study Population

This project was approved by the Hospital Universitario San Vicente de Paúl Ethics Committee. The patient group consisted of 100 consecutive patients who were diagnosed with lower-extremity DVT by duplex ultrasonography in the vascular department of the Hospital Universitario San Vicente de Paúl, Medellín, Colombia, according to previously established criteria [7]. The patient group consisted of 74 females and 26 males, with a median age of 42.9 years (11–80 years). The control group was composed of 114 healthy blood donors with no history of DVT and no hormonal treatment, who presented to the blood bank of the Hospital Universitario between September 2001 and September 2002. The control group consisted of 61 males and 53 females, with a median age of 30.6 years (17–58 years). All individuals were required to sign an informed consent.

Methods

Factor V Leiden was assessed using the method described by Ridker et al. [8]. The G20210A mutation in the prothrombin gene was identified using the method described by Poort et al. [9]. The polymorphism MTHFR C677T was genotyped using the method described by Mandel et al. [10]. APC resistance was measured using a second-generation functional assay, based on activated partial thromboplastin time (APTT) with factor V deficient plasma [11] (Test^{TM} APC^{TM} RESISTANCE V, Instrumentation Laboratory, Lexington, MA). APTT was performed in the presence and absence of an APC standard quantity, and calcium chloride was used to induce clotting. APC resistance was determined by the ratio between the clotting time, in the presence and absence of APC, and the test was considered positive when the ratio obtained was lower than 2.0 [12].

Data Analysis

Allele and genotype frequencies were compared in the two studied populations, and odds ratios (OR) were calculated with 95% confidence interval (Cornfield's confidence limits for the OR). Additionally, P values were calculated with the Mantel–Haenszel χ^2 test (Epi Info^{TM} 6 software).

RESULTS

The factor V Leiden mutation was present in heterozygous form in 10/100 (10%) patients with DVT and in 1/114 (0.9%) controls (OR = 12.56, 95% CI = 1.61–267) (Table I). APC resistance was present in 25/

99 (25.2%) cases (one patient who presented antiphospholipid syndrome was excluded; this patient blood sample did not coagulate during the APC resistance test), and in 6/114 (5.3%) controls (OR = 6.08, 95% CI = 2.23–17.4, $P < 0.001$). Ten of 25 (40%) patients and 1/6 (17%) controls with APC resistance also carried the factor V Leiden mutation. Of the remaining 15 patients with APC resistance that did not carry the factor V Leiden mutation, one had antiphospholipid syndrome and another had gastric cancer. In the five controls with APC resistance but without Factor V Leiden, possible explanations for the APC resistance were not identified; however, these patients were not systematically tested for the presence of a lupus anticoagulant.

The prothrombin G20210A mutation was found in 4/100 (4%) patients; three were heterozygous and one was homozygous. None of the individuals in the control group carried this mutation (Table I). In regard to the MTHFR C677T polymorphism, 24/100 (24%) patients and 22/114 (19.3%) controls were homozygous for the variant (OR = 1.3, 95% CI = 0.65–2.67). Forty-nine of 100 (49%) patients and 56/114 (49.1%) controls were found to be heterozygous carriers of C677T (OR = 1, 95% CI = 0.56–1.76) (Table I). The frequency of a family history of thrombosis was significantly different between patients and controls, being positive in 18/100 (18%) patients, but only 6/114 (5.3%) healthy controls (O.R = 3.95, 95% CI = 1.4–12.64).

DISCUSSION

The most frequent thrombophilic defects, found in our group of patients diagnosed with DVT, were the APC resistance phenotype and factor V Leiden, conferring a sixfold and 12-fold increased risk for thrombosis, respectively. This is in concordance with what has been reported previously in Caucasian populations [2,13–15]. Intriguingly, the APC resistance phenotype could be explained by the factor V Leiden mutation, only in 40% of the cases. Thus, the frequency of factor V Leiden among individuals with APC resistance in our population differs substantially from what has been observed in Caucasian populations, in which about 90% of the cases with APC resistance are associated with the factor V Leiden mutation. Similar results have been reported in the Mexican population, in which the factor V Leiden mutation underlied only 10% of APC resistance cases [5,16].

Several mutations, in addition to factor V Leiden, are known to be associated with the APC resistance phenotype, including the HR2 haplotype [17], the factor V Cambridge mutation [18], the factor V Hong Kong mutation [19], and mutations in the gene encoding factor VIII [20]. We have not yet tested for the presence of these mutations in our study population. However, based upon our results, we postulate that mutations in the factor V or factor VIIII genes may underlie cases of APC resistance in the Colombian population, some of which may be novel and limited to the Amerindian population.

Also, in some cases, the APC resistance not related to factor V Leiden could be acquired and can trigger DVT events, such as during pregnancy [21], hormonal treatment [22], autoimmune diseases (i.e., antiphospholipid syndrome) [23], inflammation [24], malignancy, and high levels of factor VIII [25]. Other causes of the APC resistance phenotype, such as oral contraceptives or hormonal replacement therapy, were excluded, given that none of the subjects in this study were under these treatments. Moreover, antiphospholipid antibodies and elevated factor VIII were not investigated systematically in all patients. At the time of sample collection, individuals appeared and reported to be healthy; therefore, inflammatory, autoimmune disorders and/or malignancies were not suspected, with the exception of the five patients in whom malignancy was also present, only one presented with APC resistance.

In our study, the factor V Leiden had a frequency of 10% among the patients, in contrast to the 20% frequency of factor V Leiden in nonselected Caucasian patients with DVT reported in the literature [13], which could be explained by the tri-ethnic composition of the Colombian population.

The prothrombin G20210A mutation was originally described in a Dutch population, where the prevalence found was 2.3% in healthy controls, 6.2% in nonselected patients with DVT, and 18% in selected patients with family history of DVT [9]. On the contrary, the prothrombin mutation prevalence is higher in southern Europe (prevalence of 2.6–6.5% in the Spanish healthy population) than the prevalence reported in Caucasian populations located in northern European countries [26–28]. We found four patients with the prothrombin G20210A mutation, three heterozygous and one homozygous, while none of the healthy controls had this mutation (4% of patients vs. 0% of controls, $P < 0.05$). Our results indicate that prothrombin G20210A, while of low prevalence in the general population, is associated with DVT. As a matter of fact, the prevalence of the prothrombin G20210A mutation is extremely low in the Amerindian and African populations [27–29], and its presence in the Colombian population most likely reflects Spanish admixture.

Currently, it is generally accepted that the MTHFR C677T polymorphism alone is not a risk factor for

thrombosis [30]. In the homozygous state, however, this variant can be considered a risk factor when associated with other thrombophilic conditions [31]. As we found no significant difference in the frequency of homozygotes between the patient group and healthy individuals, our results provide further support that this variant is not by itself associated with DVT.

In conclusion, we found that the presence of APC resistance, factor V Leiden, and prothrombin G20210A are associated with DVT in our population, while the MTHFR C677T polymorphism was not. Therefore, we recommend the screening for these thrombophilic alterations in Colombian patients, who are diagnosed with DVT without known acquired causes. Further investigation is required to elucidate the mechanisms of APC resistance in the absence of factor V Leiden in the Colombian (tri-ethnic) population.

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