Artigo / Article

Angiotensin converting enzyme (ACE) DD genotype: relationship with venous thrombosis

Genótipo DD da enzima conversora de angiotensina (ECA): relação com trombose venosa

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Venous thromboembolism is a common multifactorial disease associated with acquired and inherited predisposing factors. Several polymorphisms, e.g. factor V Leiden, factor II G20210A and deficiency of antithrombin, protein C and protein S, have been associated with venous thromboembolism. Angiotensin converting-enzyme affects hemostasis by decreasing fibrinolysis. Angiotensin converting-enzyme gene polymorphism, a 287 pb insertion/deletion at introns 16, is related to variations in enzyme serum levels. The DD genotype has been associated with increased risk for venous thrombosis. This study examined the frequency of the angiotensin converting-enzyme alleles I and D and their association with venous thrombosis in a group of individuals from the south of Brazil. Seventy-one patients with deep venous thrombosis and/or pulmonary thromboembolism and 71 healthy individuals were analysed in a case-control study. The angiotensin converting-enzyme ID genotyping was performed by polymerase chain reaction. The frequencies of the D allele and DD genotype were, respectively, 51.4% and 22.5% for patients, and 64.7% and 45.0% for controls. The Odds Ratio for the dominant hypothesis (DD+ID versus II genotypes) was 0. 75 (CI 95%; 0.29-1.93) and the Odds Ratio for recessive hypothesis (DD versus ID+II) was 0.35 (CI 95%; 0.16-0.78). In conclusion, our results indicate a protective effect of the angiotensin converting-enzyme DD genotype on venous thromboembolism. Rev. bras. hematol. hemoter. 2005;27(2):87-90.

Key words: *ACE; fibrinolysis; venous thromboembolism.*

Introduction

Venous thromboembolism (VTE) is a widespread multifactorial disease associated to inherited and acquired predisposing factors such as advanced age, prolonged immobilization, surgery, malignancies, antiphospholipid syndrome, oral contraceptives and hormone replacement therapy. Most polymorphisms associated to VTE lie preferentially or predominantly in genes encoding coagulant and anticoagulant proteins and include the mutations factor V Leiden, factor II (G20210A) and in genes encoding antithrombin, protein C and protein S.[1]

The renin-angiotensin system (RAS) is a complex regulator of blood pressure, water homeostasis, cardiovascular remodeling and vascular tone. This system is composed of several key proteins including angiotensinogen, angiotensin converting enzyme (ACE) and angiotensin II and its receptors affect hemostasis through different mechanisms.[2] The fibrinolytic system constitutes the endogenous defense mechanisms against intravascular thrombus formation and is activated by the presence of a fibrin clot within the vasculature. Fibrinolysis starts when plasminogen, mediated by activators, is converted to plasmin, a proteolytic enzyme.[3] Two important plasminogen activators

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in the vascular system are tissue-type plasminogen activator (t-PA) and urokinase (u-PA). Fibrinolysis is regulated by the balance between the activity of plasminogen activators and their inhibitors. Plasminogen activator inhibitor type 1 (PAI-1) is the most important physiologic inhibitor of t-PA and urokinase in plasma.[4] Clinical and experimental studies have defined the relationship between RAS and the fibrinolytic system.[3-6] Angiotensin I is converted to angiotensin II by ACE which binds to endothelial cells and stimulates the production of plasminogen activator inhibitor type I (PAI-1) thus down-regulating fibrinolysis. In addition, ACE degrades bradykinin, an important mediator of the tissue-type plasminogen activator (t-PA), which also contributes to decrease fibrinolysis, hence increasing the thrombotic risk.[4]

Polymorphism of gene encoding components of RAS, angiotensinogen, ACE and angiotensin I receptors have been associated to hypertension and myocardial infarction.[7-9] Recent studies have suggested the involvement of some polymorphisms in RAS genes in the pathogenesis of VTE.[10,11] The ACE gene polymorphism was first reported by Rigat et al, consisting of the presence (insertion-I) or absence (deletion-D) of a 287 pb fragment, at the angiotensin I converting enzyme gene (introns 16). Three genotypes can result: II, DD and ID. Their study also showed that the D allele was associated with increased serum levels of circulating enzyme.[12]

Despite the fact that most studies concerning the pathophysiologic effects of the RAS have been confined to the arterial vasculature, increasing evidence indicates that the venous system may also be affected by changes in RAS genes and its association with fibrinolysis.[2]

Previous studies have investigated the polymorphism ID of the ACE gene as a risk factor for venous thrombosis, reporting conflicting data.[10,17] The aim of this study was to determine the frequency of allele I and D in patients with VTE and investigate its association to venous thromboembolism.

Subjects and Methods

Patients and controls

This case-control study was conducted at Hospital São Lucas of Pontifícia Universidade Católica do Rio Grande do Sul (Porto Alegre, Brazil) from May 2001 to December 2003. Cases were recruited among patients attending the Cardiovascular Surgery or Hematology Ambulatory, and inpatients from the Coronary Treatment Unit. To participate in this study, patients should have suffered at least one confirmed episode of deep venous thrombosis and/or pulmonary thromboembolism. The VT diagnosis was confirmed by doppler ultrasound and PE by scan pulmonary angiography. Control subjects were recruited among Genesis Project participants (a cohort study of the Institute of Geriatrics and Gerontology – PUCRS), with no history of thrombosis, and according to patients' age, ethnicity and gender. Written informed consent was obtained from all cases and controls. This protocol was approved by the Ethics Committee for Research of the University. Patients were excluded in cases of malignant disorder, antiphospholipid syndrome or surgery.

Genotyping

Blood samples (5 mL) were collected in EDTA-K3 Vacutainer tubes. DNA was extracted using GFX Genomic kit (Amersham Pharmacia Biotec Inc) according to the manufacturer's instructions. The insertion /deletion genotyping was performed by polymerase chain reaction (PCR), according to Rigat et al,[18] modified by Ueda et al.[19] The reaction mixture (25 L) contained 20 pmol of each primer (Life Technologies, USA), 2 mM MgCl2, 200 mM dNTP mix (Life Technologies, USA), 1% DMSO, 1.75 U Taq DNA polymerase (Life Technologies, USA), 2 mM Tris-HCl pH 8.4 and 5 mM Kcl. The amplification program was 94ºC/3 min; 30 cycles of [94ºC/1 min, 52ºC/1 min, 72ºC/1 min] and 72ºC/5 min. PCR products were electrophoresed in 2% agarose gel containing ethidium bromide and analysed under UV light. Primer sequences and product sizes are shown in Table 1.

Statistical analysis

Statistical analysis was performed with EPI Info 6.0. The ACE allele and genotypic frequencies were obtained by direct count. The genotype distribution and allele frequencies were compared using the chi-square test. Odds ratio was estimated with a 95% confidence interval as a measure of risk. All statistics tests were two sided and a P value < 0.05 was considered significant.

Table 1
Primers sequence and PCR product sizes for ACE genotyping

	Primer sequence	Product size (bp)	
		D allele	I allele
Forward[1]	5'-CTGGAGACCACTCCCATCCTTTCT-3'	190	480
Reverse[1]	5'-GATGTGGCCATCACATTCGTCAGAT-3'		
Internal[2]	5'-TGGGATTACAGGCGTGATACAG-3'	-	160

[1]Rigat B et al[18]; [2]Ueda S et al[19]

Results

DNA was analysed for ACE genotypes from venous blood of 71 patients and 71 controls, 32 men (45.1%) and 39 (54.9%) women in each group. Mean age of patients was 51.2 years and for controls it was 51.7. Among patients, 46 had suffered only one episode, 25 were recurrent, 48 were DVT cases and 23 PE. The frequencies of the D allele were 51.4%

for patients and 64.7% for controls. For the DD genotype, the frequencies were 22.5% for patients and 45.0% for controls. The OR for the dominant hypothesis (DD + ID versus II genotypes) was 0.75 (CI 95%; 0.29-1.93) and for recessive hypothesis (DD versus ID + II) was 0.35 (CI 95%; 0.16-0.78). The ACE genotyping distribution and allele D frequencies and risk associated with gender for patients and controls are shown in Tables 2 and 3.

Table 2
ACE genotyping distribution

		VTE patients n (%)	Controls n (%)
ACE genotype	DD	16 (22.5)	32 (45.0)
	ID	41 (57.7)	28 (39.6)
	II	14 (19.7)	11 (15.4)

ACE: angiotensin converting enzyme; D: Deletion; I: Insertion

Table 3
D allele frequency and risk associated with gender

	Frequency		Dominant effect (DD+ID/II)		Recessive effect (DD/ID+II)	
	%	OR (CI95)	%	OR (CI95)	%	OR (CI95)
Patients	51.4	0.57 (0.35-0.95)	80.2/19.8	0.75 (0.29-1.93)	22.5/77.5	0.35 (0.16-0.78)
Controls	64.7		84.5/15.5		45.0/55.0	
Males						
Patients	50.0	0.68 (0.32-1.46)	78.0/22.0	1.19 (0.32-4.40)	21.8/78.2	0.57 (0.10-1.21)
Controls	48.7		75.0/25.0		43.7/56.3	
Females						
Patients	64.0	0.49 (0.24-1.00)	82.0/18.0	0.38 (0.07-1.84)	23.0/77.0	0.35 (0.12-1.03)
Controls	69.2		92.0/8.0		46.2/53.8	

OR: odds ratio; CI95: confidence interval 95%

Discussion

The interaction of RAS with hemostasis components common to venous and arterial diseases suggests a relationship of genetic polymorphisms, the coagulation system and VTE. ACE is related to hypofibrinolysis by both increasing the PAI-1 expression and decreasing t-PA production.

In the present study, 22.5% of cases and 45% of controls have the ACE DD genotype. The overall OR for the dominant hypothesis (DD + ID versus II genotypes) was 0.75 (CI 95%; 0.29-1.23; P= 0.51) and it was 0.35 for the recessive hypothesis (DD versus ID +II) (CI 95%; 0.16-0.78; P= 0.004). This finding suggests a protective effect of the ACE DD genotype on VTE.

Dilley et al, studying African-Americans with venous thrombosis demonstrated a moderate increase of venous thrombosis risk in male patients with the DD genotype (OR 2.8), but no increased risk for women patients.[11] No correlation between ACE genotype and venous thrombosis was found by Ordoñez et al in a study conducted with patients under coumarin therapy from a Caucasian population in the north of Spain.[13] Jackson et al, in another case-control study conducted of more than 500 unselected patients, the ID polymorphism in the ACE gene was not a risk factor for venous thromboembolism.[14] Della Valle et al, analysed the genetic profiles of 38 patients who had a postoperative symptomatic pulmonary embolus or proximal deep venous thrombosis following a joint arthroplasty and 241 control subjects, without thrombosis. No difference was observed between both groups. These results suggest that there is no association between the presence of the D allele and increased risk of symptomatic thromboembolic events following total hip or knee arthroplasty.[15] In contrast, it was reported by Philip et al. that the deletion polymorphism of ACE gene is a significant risk factor for venous thrombosis following hip replacement surgery. The homozygous DD genotype increased the thrombotic risk more than 11-fold (OR 11.7 CI 95% 2.3-84.5), and heterozygous ID genotype increased the risk approximately 5-fold.[10] It is important to point out that the study of Phillip et al was conducted with postoperative patients and there is a known influence of endothelial damage induced by orthopedic surgery in the pathogenesis of thrombosis, which may interfere in the analysis of genetic risk factors. On the other hand, patients studied by Della Valle, also underwent hip surgery and no association was found.

Recently, Fatini et al showed a significant association between ACE DD genotype and venous thromboembolism adjusted for acquired and hemostasis risk factors ($p<0.0001$). This study included 336 patients, 40% idiopathic VTE and 60% secondary VTE. They concluded that ACE DD genotype increases the risk of venous thromboembolism in subjects apparently without predisposing factors and thrombophilic alterations, and in subjects in whom a thrombogenic condition occurs.[16] In contrast, Wells et al, in a case-control study that prospectively enrolled consecutive patients with at least one objectively confirmed idiopathic VTE (n=300), concluded that the ACE DD genotype is protective against idiopathic venous thromboembolism (0.66).[17]

Results of publications on the relationship of ACE ID genotype and VTE presented above are contradictory and some limitations (e.g., the broad spectrum of patients enrolled) are found. Most of all, they evaluate groups of patients with different profiles, therefore a comparative analysis of the reported results would be hard to perform. Our study was similar to, and indeed in accordance with, the study devised by Wells et al., both excluding VTE patients with malignancy, surgery, and antiphospholipid syndrome. This represented a

limiting factor and accounted for our restricted sample size. Individuals from the control group were selected among a healthy population from the same geographic area of patients, according to patients' age, ethnicity and gender. We consider those a more suitable control group, once their medical history excluded any thrombotic event. It is important to point out that most of our patients were Caucasian and so probably our results do not apply to other racial groups.

In conclusion, our results suggest that the ACE DD genotype do not represent a risk factor for venous thrombosis and may exert a protective effect on VTE. Further studies are needed, possibly conducted with an extended population, in order to confirm our findings.

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Resumo

O troemboembolismo venoso (TEV) é uma doença multifatorial associada com fatores de risco adquiridos e hereditários. Vários polimorfismos, tais como fator V de Leiden, mutação G20210A da protrombina e as deficiências de proteína C, proteína S e antitrombina são considerados fatores de risco para TEV. A enzima conversora da angiotensina (ECA) afeta a hemostasia diminuindo a fibrinólise. O polimorfismo no gene da ECA, caracterizado pela inserção/deleção de um fragmento de 287 pb no intron16, está relacionado a variações nos níveis séricos da enzima. O genótipo DD foi associado com aumento de risco para TEV. Este estudo examinou a freqüência dos alelos I e D e a sua associação com trombose venosa em um grupo de indivíduos do Sul do Brasil. Foram analisados 71 pacientes com trombose venosa profunda e/ou tromboembolismo pulmonar e 71 indivíduos sem história de trombose. A genotipagem foi realizada através da reação em cadeia da polimerase. As freqüências do alelo D e do genótipo DD foram, respectivamente, 51,4% e 22,5% para os pacientes, e 64,7% e 45,0% para os controles. A razão de chance (odds ratio = OR) para a hipótese dominante (genótipos DD+ID versus genótipo II) foi 0,75 (IC 95%;0,29-1,93) e a OR para a hipótese recessiva (genótipo DD versus genótipos ID+II) foi 0,35 (IC 95%; 0,16-0,78). Concluindo, nossos resultados sugerem que o genótipo DD não representa um fator de risco para TEV e pode exercer um efeito protetor para trombose venosa. Rev. bras. hematol. hemoter. 2005; 27(2):87-90.

Palavras-chave: *ECA; fibrinólise; tromboembolismo venoso.*

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