The β-fibrinogen -455 G>A gene polymorphism is associated with peripheral vascular injury in systemic sclerosis patients

Sirs,

A number of single nucleotide polymorphisms (SNP)s have been reported to increase the risk of systemic sclerosis (SSc) and of some distinct manifestations of this condition (1, 2).

Although an altered coagulation/fibrinolysis balance has been implicated in the pathophysiology of SSc (3), SNPs known to promote arterial and venous thrombosis in the general population, *i.e.* factor V G1691A (Leiden), prothrombin G20210A and β-fibrinogen -455 G>A, have never been investigated in SSc patients.

We have looked for these 3 SNPs in 47 SSc patients (42 women and 5 men, aged 22-67 years, all meeting the ACR classification criteria for the disease (4)) and 66 ethnically matched healthy controls (62 women and 4 men, aged 25-65 years). The study protocol was approved by the local ethics committee, and all subjects gave their informed consent.

SSc patients were characterised for clinical and serological subset, and organ/system involvement as reported elsewhere (5, 6). Factors promoting peripheral vascular injury, namely, smoking habit, diabetes mellitus, anti-phospholipid antibodies and cryoglobulins were ruled out.

Genomic DNA samples were obtained from the peripheral blood of SSc patients and controls. SNPs were detected by PCR amplification using biotinylated primers, hybridising the amplification products to a test strip containing allele-specific oligonucleotide probes, and finally revealing bound biotinylated sequences by streptavidin-alkaline phosphatase and color substrates (ViennaLab Labor-diagnostika GmbH, Austria).

The frequency of the 3 prothrombotic SNPs did not differ significantly between SSc patients and controls: 2.1 *vs.* 4.5% for factor V G1691A ($p>0.05$); 10.6 *vs.* 9.1% for prothrombin G20210A ($p>0.05$); 40.4 *vs.* 48.5%, for β-fibrinogen -455 G>A ($p>0.05$). However, the β-fibrinogen -455 A allele was more frequent in SSc patients with digital lesions (pitting scars/ulcers) than in those with uncomplicated Raynaud's phenomenon (13/17 *vs.* 6/30) (OR=13.000; 95% CI 3.098 to 54.557; $p=0.0002$) (Table I), even though all patients were under antiplatelet/anticoagulant and peripheral vasodilatory treatment. In fact, 41/47 (87.2%) patients were taking calcium channel blockers, and 6/47 (12.8%) second line vasodilators (α-blocker or ACE-inhibitor compounds).

A heterozygous genotype G/A was detected in all patients. Moreover, the 19 SSc patients carrying the β-fibrinogen -455 G/A genotype showed an increase in fibrinogen plasma levels that paralleled the severity of peripheral vascular involvement as evaluated by Medsger's severity scale (6): 291.83±50.90mg/dl in patients with a score of 1 (patients with Raynaud's phenomenon requiring vasodilators) *vs.* 301.38±54.55mg/dl in patients with a score of 2 (patients with digital pitting scars) *vs.* 359.2±75.66 mg/dl in patients with a score of 3 (patients with digital ulcers). No patient had a score of 0 (no Raynaud's phenomenon or Raynaud's phenomenon not requiring vasodilators) or 4 (digital gangrene).

The -455 G>A polymorphism in the proximal promoter region of the β-fibrinogen gene is reported to be an independent risk factor for thrombosis in both homo- (A/A) and heterozygosity (G/A) and to increase plasma fibrinogen levels (7, 8). Fibrinogen increases blood viscosity, erythrocyte aggregation, vasoreactivity and endothelial permeability (9), thereby promoting intravascular and parietal thrombosis in individuals with an underlying vascular injury, as occurs in SSc patients (10).

Our study illustrates a hitherto unknown association between the β-fibrinogen -455 A allele and peripheral vascular damage in SSc patients. However, given the low number of patients investigated and the cross-sectional nature of the study, we cannot draw any conclusion about the prevalence and the significance of prothrombotic SNPs in SSc patients. Nevertheless, our preliminary data suggest that inherited thrombophilia may play a role in microvascular involvement in SSc, thereby providing the rationale for larger studies on this topic.

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Table I Frequency of factor V G1691A, prothrombin G20210A, β-fibrinogen -455 G>A SNP in the investigated 47 SSc patients according to the baseline characteristics: sex; clinical and serological subset; peripheral vascular, pulmonary, heart and kidney SSc-related involvement. Data are reported as numbers (percentages).

Prothrombotic SNP	Sex		Clinical subset		Serological subset		Organ/system involvement							
	Female	Male	dcSSc	lcSSc	ACA	Anti-Scl70	Peripheral vascular		Pulmonary		Heart		Kidney	
							Yes	No	Yes	No	Yes	No	Yes	No
	42 (89.4)	5 (10.6)	10 (21.3)	37 (78.7)	28 (59.6)	19 (40.4)	17 (36.2)	30 (63.8)	38 (68.1)	9 (31.9)	29 (61.7)	18 (38.3)	0	47 (100)
Factor V G1691A	1/42 (2.4)	0	0	1/37 (2.7)	0	1/19 (5.3)	0	0	1/38 (2.6)	0	0	1/18 (5.6)	0	1/47 (2.1)
Prothrombin G20210A	4/42 (9.5)	1/5 (20)	0	5/37 (13.5)	4/28 (14.3)	1/19 (5.3)	1/17 (5.9)	0	5/38 (13.2)	0	4/29 (13.8)	1/18 (5.6)	0	5/47 (10.6)
β-fibrinogen -455G>A	17/42 (40.5)	2/5 (40)	5/10 (50)	14/37 (37.8)	11/28 (39.3)	8/19 (42.1)	13/17* (76.5)	6/30* (20)	17/38 (44.7)	2/9 (22.2)	14/29 (48.3)	5/18 (4.6)	0	19/47 (40.4)

SNP: single nucleotide polymorphism; SSc: systemic sclerosis; dcSSc: diffuse cutaneous SSc; lcSSc: limited cutaneous SSc; ACA: anticentromere antibodies; anti-Scl70: anti-topoisomerase I antibodies. *$p<0.0002$.

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