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Etrurians vs Greeks: May *ACE* I/D polymorphism still be considered as a marker of susceptibility to SSc?

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ABSTRACT

Objective. *SSc is characterized by immune dysfunction and microvascular involvement. A different genetic background may determine a different polymorphic allele frequency between different populations, and data from literature reported conflicting results about the role of genetic components in predisposing to the disease. We carried out this study in order to compare the* ACE *I/D polymorphism genotype distribution and alleles frequency in two different populations from the Mediterranean area.*

Methods. *Forty-eight Italian and 41 Greek SSc patients compared with 112 Italian and 93 Greek controls, have been studied. The* ACE *I/D polymorphism has been analysed.*

Results. *The genotype distribution and allele frequency were in Hardy-Weinberg equilibrium for Italian and Greek SSc patients and controls. Among the Italian patients a significantly higher* ACE *D allele frequency than in the controls was found, whereas among the Greeks a higher prevalence was observed in the healthy subjects. A significant difference in* ACE *D allele frequency between Italian and Greek controls was observed (p = 0.04).* ACE *D allele was associated to the predisposition to SSc in Italians, but not in Greeks.*

Conclusion. *We confirm that Italian SSc patients have a higher* ACE *D allele frequency that is not present in the Greek patients. Thus, the two populations living in different Mediterranean areas and resulting from the Mediterranean civilization, do not show the same* ACE*-gene related allele frequencies. Other populations of the Mediterranean area must be investigated by using unlinked genetic markers to verify the homogeneity of the genetic background, and to test for a "true" difference in their ethnic origin.*

Introduction

Systemic sclerosis is characterized by immune dysfunction, fibroblast overproduction of collagen, and microvascular involvement. The main pathologic hallmark of the disease is endothelial derangement, which is the main cause of vascular features, such as Raynaud's phenomenon and pulmonary hypertension (1). These clinical features are due to the injury to the microvascular endothelium that is followed by smooth muscle cells migration in the intima (1). The pathological alterations of the vessel wall in SSc are strikingly similar to the modification detected in atherosclerotic lesions (2), and it is now evident that SSc is also characterized by accelerated macrovascular disease (2). This evidence has led to studies of the mechanisms implicated in the injury of the vessel wall that may contribute not only to the microvascular involvement but also to the development of a precocious macrovascular disease (3). The endothelium is thought to link the fibrinolytic system and the renin-angiotensin system (RAS). *In vivo* and *in vitro* studies show that the activation of the RAS may modify the balance of the fibrinolytic system. Angiotensin-converting enzyme (ACE), the key enzyme in the RAS, converts angiotensin I into angiotensin II, a potent vasoconstrictor. In intron 16 of the gene encoding for ACE on chromosome 17q23, an insertion/deletion (I/D) polymorphism consisting of the presence or absence of a 287-base pair (bp) Alu repeat, has been identified (4). Actually, the ACE I/D polymorphism has been related to ACE enzyme levels (5), with a dose-dependent effect, and data from experimental studies reported a functional role for this polymorphism in modulating the angiotensin II levels (6), and an increased mRNA expression in white blood cells from subjects carrying the D allele in comparison to subjects carrying the I allele has been found (7).

Our previous data documented a high ACE D allele frequency in SSc patients and suggested that the ACE D allele of the I/D polymorphism was associated with an increased risk of SSc (8). The results showing that the possession of at least one D allele was associated with an increased risk of malignant vascular involvement. This has led to suggest that I/D polymorphism may be a useful genetic marker to identify those SSc patients who risk developing a severe vascular disease frequently leading to gangrene (9). In American

SSc patients, Assassi *et al.* (10) did not find an association between ACE I/D polymorphism and SSc. In SSc Korean patients, an increase of HLA DR1*15 alleles was found but no difference in ACE I/D gene polymorphisms between SSc and controls was detected (11). Recently, we supported the role of ACE I/D polymorphism as predisposing factor to SSc and demonstrated its involvement in accelerated macrovascular disease (12) as demonstrated by increase of carotid intima media thickness (12). In SSc, not only endothelial but also downstream mechanisms linked to ACE I/D polymorphism damage may significantly contribute to accelerated macrovascular disease. In fact, the ACE D allele affects plasma ACE levels (5), thereby regulating the production of angiotensin II and the degradation of bradykinin, and contributing to mechanisms involved in the induction and maintenance of vessel wall modification and enhancement of atherosclerosis. In order to verify if the association of ACE I/D polymorphism and SSc is influenced by the geographical origin, we compared the ACE I/D polymorphism genotype distribution and allele frequency of an Italian population with another Mediterranean population from a different geographical area, such as the Greeks.

Materials and methods

Two groups of SSc patients from different geographical areas were studied: 48 Italian patients, from Tuscany (42 females and 6 males, mean age 59.75 ± 12.09) and 41 Greek patients from Peloponnese (39 females and 2 males, mean age 49.17 ± 12.5). The control group consisted of 112 Italian subjects recruited among the staff at the University of Florence in Tuscany (70 females and 42 males, mean age 58.35 ± 13.38), and 93 Greek subjects from Peloponnese (89 females and 6 males, mean age 45.76 ± 8.8). All the subjects enrolled in the study, both the patients and the control group, were unrelated to each other. Italian and Greek patients and controls signed an informed consent.

The *ACE* I/D polymorphism was analysed as previously described (8). The statistical analysis was performed by using the SPSS (Statistical Package for Social Sciences, Chicago, USA) software for Windows (Version 10.0). The *ACE* gene alleles frequencies were obtained by direct count; the genotype distribution and allele frequency were compared by using the χ^2-test. The χ^2-test was used to test for deviation from Hardy-Weinberg equilibrium. The role of *ACE* I/D polymorphism as predisposing factor to SSc was analysed by univariate logistic regression analysis by using a dominant model of inheritance (eg, considering subjects homozygotes and heterozygotes for the variant). Odds ratio (OR) with 95% confidence interval was determined. A p-value < 0.05 was considered to indicate statistical significance.

Results

ACE I/D polymorphism genotype distribution and allele frequencies of both populations are reported in Table I. The genotype distribution of the *ACE* I/D polymorphism was in Hardy-Weinberg equilibrium for both populations analysed, so excluding laboratory inaccuracies and selection bias.

Among the Italians, a significant higher prevalence of *ACE* D allele was found in the patients in comparison to the controls. On the contrary, among the Greeks a higher, but not significant, prevalence of the *ACE* D allele was observed in the controls. As far as the Italian and Greek controls are considered, we observed a significant difference in ACE D allele frequency, but not in genotype distribution between the two populations (χ^2 = 4.11, p = 0.04 and χ^2 = 5.09, p = 0.08, respectively).

The *ACE* D allele was significantly associated to the disease (OR DD+ID vs. II=2.92 95%CI 1.13-7.54, p = 0.02) in the Italian population, but not in the Greek SSc patients (OR DD+ID vs. II=0.59 95%CI 0.24-1.47, p = 0.2).

Discussion

This study, comparing the *ACE* I/D polymorphism allele frequencies from two populations of the Mediterranean area, shows a difference in *ACE* D allele frequency between the healthy Italian population from Tuscany and from Peloponnese. Moreover, our results show that the D allele does not represent a susceptibility factor to SSc in Greeks, differently from our previous results in Italians showing that *ACE* D allele influenced the susceptibility to SSc (8). The finding of a different prevalence of the *ACE* D allele not only in patients, but in particular, in healthy subjects from Greece in comparison to Italians, suggest that the ethnic origin of these two populations may have different predisposition to SSc. Most studies have suggested that ethnic factors impact significantly on complex diseases. As far as SSc is con-

Table I. Genotype distribution and allele frequency of the ACE I/D polymorphism in the Italian and Greek populations.

	Italian SSC patients (n = 48) (%)	Controls (n = 112) (%)	p
ACE genotype			
ACE DD	20 (41.7)	31 (27.7)	
ACE ID	22 (45.8)	48 (42.8)	
ACEII	6 (12.5)	33 (29.5)	0.04*
ACE D frequency	0.64	0.49	0.01**
	Greek SSC patients (n = 41) (%)	**Controls (n = 93) (%)**	**p**
ACE genotype			
ACE DD	10 (24.4)	32 (24.4)	
ACE ID	21 (51.2)	46 (49.5)	
ACEII	10 (24.4)	15 (16.1)	0.4*
ACE D frequency	0.50	0.59	0.16**

*Genotype distribution (χ^2 = 6.09 in Italians; χ^2 = 1.97 in Greeks).
**Allele frequency (χ^2 = 6.47 in Italians; χ^2 = 1.93 in Greeks).

cerned, epidemiological studies, carried out in population of different origin, indicate that blacks are affected twice as frequently as whites, and Japanese patients have a lower prevalence than Caucasians. Nevertheless, are these two Mediterranean populations "really"different in the genetic background? An answer could benefit from an adjustment by testing a set of unlinked genetic markers in the study populations to directly determine if there is a substantial difference between the estimated ancestry of the sampled cases and controls.

This study has two limitations: the first is the small sample size of the populations analysed, the second is the risk of stratification, which is a common bias in the case-control studies, and which arises when there is a sufficiently large difference in both ethnic admixture between cases and controls. In conclusion, by analysing the prevalence of the *ACE* I/D polymorphism, we observed that two populations living in two Mediterranean areas, and resulting from the Mediterranean civilization, have different ACE gene-related allele frequencies. Ethnic differences in allele frequency may make ACE I/D polymorphism more significant in some populations with respect to others (13). The results obtained in this study on two different populations, with a different genetic background, question our previous data (12) thus complicating the "ACE paradox" (14) in SSc.

ACE genotyping has been used as a tool to investigate the role of tissue ACE in human disease pathogenesis. The discrepancy of the results of ACE genotype associations with myocardial infarction (15) and now with SSc, was described. This evidence raises the main question that, likely genotyping is a dead end for understanding the real significance of ACE role and contribution to a disease such as SSc. Further studies, addressing genotyping and phenotyping ACE activity in order to understand the whole scenario from genes to tissue and circulating levels in SSc are warranted.

In the future, Italians and Greeks may be tested with unlinked genetic markers in order to verify the homogeneity of the genetic background in both populations. Indeed, the analysis of the genetic polymorphisms should be spread to other Mediterranean populations in order to test for a "real" difference in their ethnic origin.

The number of investigated subjects renders any definite conclusions difficult to make. Thus, our results should be interpreted as preliminary, and a larger study is required to confirm the current finding and to decipher the contribution of genetic variation to SSc based upon a different ethnic origin of the investigated populations.

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