

Angiotensin-Converting Enzyme Gene Does Not Contribute to Genetic Susceptibility to Systemic Sclerosis in European Caucasians

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ABSTRACT. Objective. To determine whether angiotensin-converting enzyme (ACE) polymorphisms including I/D and 2 single-nucleotide polymorphisms (SNP) affect susceptibility to systemic sclerosis (SSc) in a large French Caucasian population.

Methods. A case-control study was performed in 494 patients with SSc and 280 healthy controls for I/D polymorphism. Two supplementary exonic SNP of ACE gene (rs4309, rs4362) were genotyped in 659 patients with SSc and 511 matched healthy controls. Among the whole SSc population, 453 (67%) patients with SSc had the limited cutaneous subtype, 47 (7%) had precapillary pulmonary arterial hypertension, 209 (32%) had digital ulcers, and 10 (1.5%) had renal crisis. A combined analysis of the available results for ACE I/D genotypes in Caucasians was also performed.

Results. There was no association between the 3 polymorphic markers and SSc for allelic and genotype frequencies. No association was observed for the different vascular subsets of the disease. Haplotype analyses did not detect any association. The lack of association for ACE I/D was confirmed by the combined analysis.

Conclusion. These results in a large cohort of European Caucasian patients with SSc do not support that the ACE gene is implicated in the pathogenesis of SSc and its vascular damage. (First Release Dec 15 2008; J Rheumatol 2009;36:337–40; doi:10.3899/jrheum.080622)

Key Indexing Terms:

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Although the pathogenesis of systemic sclerosis (SSc) remains unclear, microvascular abnormalities have been reported as an early key step of the disease. In SSc renal crisis, a local activation of the renin-angiotensin system (RAS) has been observed¹. Moreover, angiotensin-converting enzyme (ACE) inhibitors were found to dramatically improve the outcome of the renal crisis in SSc². The RAS is closely implicated in macrovascular disease favoring vaso-

constriction; in SSc, prevalence of macrovascular involvement remains a matter of debate^{3,4}.

SSc is a multifactorial disease and it is believed that both genetic and environmental factors contribute to disease susceptibility⁵. ACE, a key enzyme in the RAS, is encoded by the ACE gene, mapping to the 17 (17q 23) region. An insertion/deletion (I/D) polymorphism has been reported associated not only with the level of ACE but also with endothe-

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lial disturbances⁶. In SSc, the question of an association of ACE I/D has been studied in different populations, with conflicting results. Our aim was to assess whether polymorphisms in *ACE* gene affect susceptibility to SSc in large European Caucasian populations.

MATERIALS AND METHODS

First, we included 774 consecutive unrelated subjects for the ACE I/D genotyping according to a published procedure⁶, comprising 494 patients with SSc classified according to LeRoy, *et al*⁷ and 280 healthy age- and sex-matched controls (mean age 66 ± 16 yrs; 75% were female). Then, 1170 subjects, 659 patients with SSc and 511 healthy matched controls (including those of the first set), were included for the genotyping of 2 supplementary ACE SNP (rs4309 and rs4362) using the KASpar Genotyping system (KBioscience)⁸.

All subjects were of European Caucasian origin, defined by all 4 grandparents being French Caucasian. The Ethics Committee of Cochin Hospital approved our study and all the individuals gave written informed consent.

We thereafter conducted a combined analysis restricted on Caucasian populations; literature search was performed on articles published and expanded on Medline, EMBASE, and European League Against Rheumatism/American College of Rheumatology congress abstract archives. We obtained 4 independent populations including our study. We extracted the data from contingency tables of Fatini, *et al*⁹, Assassi, *et al*¹⁰, and Guiducci, *et al*¹¹ for ACE I/D genotypes.

Statistical analyses. The Hardy–Weinberg equilibrium was investigated with a chi-squared test with 1 degree of freedom. Power calculations were driven through an asymptotic non-central chi-squared approach and provided a power of 80% to detect the association between SSc for a genotype of 30% frequency with an OR of 1.5 at the 5% significance level. Finally, ACE haplotypes were constructed using the maximum likelihood procedure. Fisher's exact test was used to compare alleles and genotype frequencies using R software version 2.6.0. Odds ratios (OR) were calculated with the most frequent homozygous genotype or allele as reference.

A combined analysis was conducted via logistic regression models, adjusted for each of the 4 Caucasian populations studied.

RESULTS

The demographic data and disease characteristics of patients with SSc in both sets are detailed in Table 1. All the 3 polymorphisms were in Hardy–Weinberg equilibrium for the control group. Frequencies of alleles and genotypes for the 3 markers are provided in Table 2. The frequency of D allele of insertion polymorphism and the minor allele for, respectively, the 2 SNP (rs4309 and rs4362) was not different in SSc and in controls, with, respectively, 54% versus 56% ($p = 0.56$, OR 0.94, 95% CI 0.77–1.16) for ACE I/D, 41% versus 39% ($p = 0.33$, OR 1.09, 95% CI 0.92–1.30) for rs4309, and 45% versus 44% ($p = 0.79$, OR 1.02, 95% CI 0.86–1.21) for rs4362. The frequency of genotypes carrying at least one D allele for ACE I/D and one minor allele for SNP was similar in SSc patients compared to controls (Table 2). Regarding SSc and subgroup analyses, especially for those with vascular involvement, we did not detect any allelic and genotypic difference. There was neither overall association ($p = 0.65$) nor association for common haplotypes with disease status (data not shown).

The combined analysis (Figure 1) showed that the frequency of D allele among the SSc cases ($n = 684$; Table 2,

Table 1. Characteristics of the French Caucasian cohort of patients with systemic sclerosis.

Patients, n (%)	SSc cohort for ACE ID (n = 494)	SSc cohort for ACE SNP (n = 659)
Age, yrs \pm SD	57 \pm 14	59 \pm 11
Sex, female	416 (84)	574 (87)
Disease duration, yrs \pm SD	12 \pm 9	11 \pm 8
Diffuse cutaneous subtype	163 (33)	216 (33)
Pulmonary fibrosis	194 (39)	271 (41)
DLCO/VA < 75%	160 (32)	291 (44)
Pulmonary arterial hypertension	34 (7)	47 (7)
Digital ulcerations	162 (33)	203 (31)
Renal crisis	3 (1)	10 (1.5)
Positive antitopoisomerase I Abs	119 (24)	163 (25)
Positive anticentromere Abs	179 (36)	270 (41)

SSc: systemic sclerosis; ACE: angiotensin-converting enzyme; DLCO/VA: diffusing capacity for carbon monoxide divided by alveolar volume; Abs: antibodies; SNP: single nucleotide polymorphism; SD: standard deviation.

combined populations) did not differ from that found among the controls ($n = 563$): 56% vs 54%, respectively ($p = 0.48$, OR 0.89, 95% CI 0.65–1.22). Neither was the frequency of genotypes carrying at least one D allele different among patients and controls ($p = 0.89$, OR 1.02, 95% CI 0.79–1.29).

DISCUSSION

In SSc, vascular involvement has a major effect on the prognosis¹². Numerous arguments support a role of RAS in SSc: (1) ACE is suspected to play a key role in renal crisis; (2) SSc is characterized by vasospasm and ACE participates in regulation of vascular tone; and (3) genetic association between ACE I/D and SSc has been suggested.

Conflicting data have been reported: an association was detected between D allele of ACE I/D in an Italian cohort⁹ but this was not replicated within North American¹⁰, Greek¹¹, or Korean populations¹³. These discrepancies may be explained by underpowered studies and by population stratification because of a heterogeneous genetic background. To avoid these biases we have focused on European Caucasians and recruited a large sample size providing a strong power (> 80%). Our results show no association between D allele or genotype carrying at least one D allele (DD and ID) or the 2 SNP investigated and SSc. The validity of our results is further supported by our control group results that are close to those reported in HapMap (NCBI database) and also in a recent series¹⁴. Our combined analysis for ACE I/D polymorphism in Caucasians provides further evidence of the absence of involvement of the ACE I/D in SSc. One limitation may be that we did not perform macrovascular assessment⁶ in our large series, although digital ulcers may somehow represent patients with middle-size/small artery involvement¹⁵. Another limitation comes

Table 2. Genotypic and allelic frequencies of the three ACE polymorphisms.

ACE polymorphisms	SSc Sample size	1/1 n (%)	1/2 n (%)	2/2 n (%)	F (2)	Controls Sample size	1/1 n (%)	1/2 n (%)	2/2 n (%)	F (2)	p for genotypes	OR (Reference: genotype 1/1)
ACE ID (I/D) minor allele (2) = I	494	148 (30)	242 (49)	104 (21)	0.46	280	88 (31)	137 (49)	55 (20)	0.44	0.87	OR _{1/2} = 1.05 [0.75; 1.47] OR _{2/2} = 1.12 [0.74; 1.71]
ACE ID (I/D) minor allele (2) = I (combined cohorts)	684	209 (31)	342 (50)	133 (19)	0.44	563 (combined cohorts)	174 (31)	265 (47)	124 (22)	0.46	0.63	OR _{1/2} = 1.06 [0.81; 1.39] OR _{2/2} = 0.87 [0.63; 1.21]
Pooled analysis rs4309 (C>T) minor allele (2) = T	640	229 (36)	296 (46)	115 (18)	0.41	452	169 (37)	213 (47)	70 (16)	0.39	0.57	
rs4362 (T>C) minor allele (2) = C	646	199 (31)	307 (47)	140 (22)	0.45	442	135 (31)	217 (49)	90 (20)	0.45	0.64	

ACE: angiotension converting enzyme; F (2): minor allele frequency; OR: odds ratio.

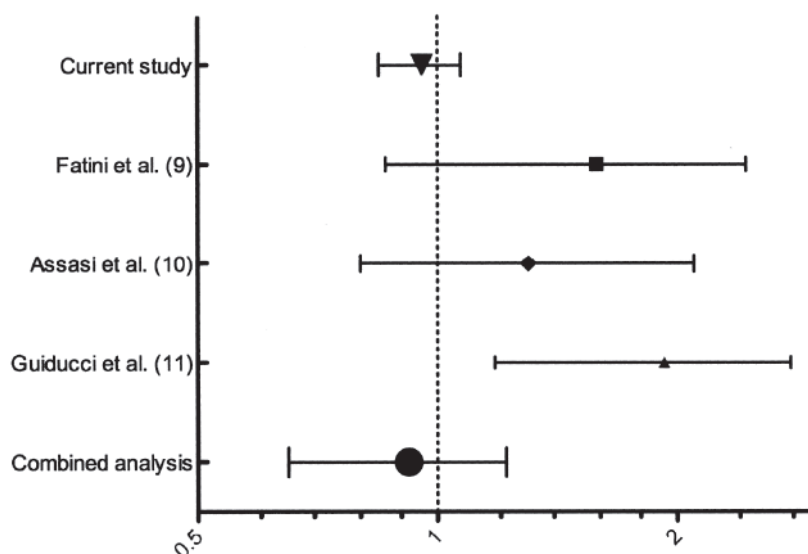


Figure 1. Combined analysis of the 4 Caucasian case-control studies of systemic sclerosis (SSc) and the ACE I/D genotypes. Results from our study and those conducted by Fatini, *et al*⁹, Assasi, *et al*¹⁰, and Guiducci, *et al*¹¹ were analyzed by logistic regression. Values are the odds ratio and 95% confidence interval (plotted on a logarithmic scale).

from the small number of patients with renal crisis; thus ACE polymorphisms will have to be investigated in a larger cohort of SSc patients with renal crisis.

The genotyping of 3 ACE gene polymorphisms in a large cohort of European Caucasian patients with SSc did not allow us to detect any allelic, genotypic, or haplotypic associations with the disease or its main vascular complications.

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