Angiotensin-converting enzyme I/D polymorphism and macrovascular disease in systemic sclerosis


Objective. Systemic sclerosis (SSc) is characterized by microvascular and macrovascular alterations. The D allele of the ACE I/D polymorphism is known to be associated with an increased incidence of atherosclerosis and has been recently proposed as associated with increased risk of SSc. This study evaluates the relationship between intima-media thickness (IMT), ankle-brachial pressure measurements (ABPI) and ACE I/D polymorphism in SSc patients.

Methods. According to the presence of ACE D allele (analysed by PCR), 53 SSc patients (47 females and 6 males; median age was 60.4 ± 10.68 yrs; range 40–75 yrs) were divided in carriers of the D allele (DD + ID) (n = 46) and carriers of the I allele (II) (n = 7). In these patients, IMT and ABPI (calculated as the posterior tibial artery pressure (mmHg) divided by the brachial pressure) were obtained. Forty-three healthy controls (40 women and 13 men; median age 56.3 ± 10.23; range 40–70 yrs) of the same ethnicity were recruited.

Results. SSc patients had IMT significantly higher than controls (0.85 ± 0.03 vs 0.68 ± 0.01; P < 0.03). No significant differences (P > 0.3) in ABPI values between patients (1.018 ± 0.10) and controls (1.091 ± 0.11) were found. SSc patients with ACE DD and ID genotype showed an IMT significantly greater (0.89 ± 0.03) than those carrying the II genotype (0.61 ± 0.01) (P < 0.04). ABPI was not different among ACE gene genotypes.

Conclusion. Our findings confirm an increased prevalence of macrovascular disease in SSc patients and show that IMT is greater in patients carrying the ACE DD and ID genotype in comparison with II homozygotes. This suggests that, in SSc, the presence of ACE D allele may predispose to an involvement of the macrovascular system.

Keywords: Systemic sclerosis, Macrovascular disease, ACE polymorphism, Intima media thickness.

Systemic sclerosis (SSc) affects the skin and internal organs leading eventually to tissue fibrosis. However, one of the main hallmark of the disease is the microvascular involvement that leads to the impairment of the blood flow to the extremities and vital organs. Moreover, in the last decade an increased prevalence of macrovascular involvement, in particular of the carotid artery has also been described in SSc [1–5] (Table 1).

Large observational studies and atherosclerosis (AS) regression trials have established that intima-media thickness (IMT) of the carotid arteries, is a valid surrogate marker for the progression of AS disease [6]. Furthermore, an increased IMT is considered as a surrogate of more generalized AS and a predictive factor for the occurrence of cardiovascular events such as stroke and myocardial infarction. IMT is being used increasingly as a mark of the disease is the microvascular involvement that leads to tissue fibrosis. However, one of the main hallmarks of the disease is the microvascular involvement that leads to the impairment of the blood flow to the extremities and vital organs. Moreover, in the last decade an increased prevalence of macrovascular involvement, in particular of the carotid artery has also been described in SSc [1–5] (Table 1).

The relationship between macrovascular disease and renin-angiotensin-system (RAS) involved in sodium homeostasis, maintenance of vascular tone and cardiovascular remodelling has been demonstrated. Angiotensin-converting enzyme (ACE), a key component of the RAS, converts angiotensin I into the vasoconstrictor angiotensin II [8], degrades bradykinin (a potent vasodilator), reduces the production of tissue plasminogen activator and stimulates prostacyclin and nitric oxide release, thereby regulating fibrinolysis and platelet activation and aggregation [9, 10]. Several studies reported that pharmacological inhibition of ACE reduced both AS in cholesterol-fed rabbits and aortic AS in rabbits with heritable hyperlipidaemia, suggesting a role for ACE in the development of atherosclerotic macrovascular disease [11]. A potential mechanism by which ACE may enhance the atherosclerotic-related thrombosis development is the effect of ACE on the fibrinolytic balance. Experimental and clinical studies have shown that angiotensin II is involved in platelet aggregation and increase of tissue factor expression in coronary artery lesion in patients with acute coronary syndromes [12]. In SSc, several modifications of the pathway leading to fibrin deposition and to activation of fibrinolytic system have been reported [13].

Genes encoding for components of RAS regulate ACE levels, as well as angiotensin II expression and function. An insertion/deletion (I/D) polymorphism in intron 16 of ACE gene, on chromosome 17q23, consisting of the presence or the absence of a 287 bp Alu repeat, has been identified [14]. This polymorphism consists of three genotypes: DD and II homozygotes, and ID heterozygote. In Caucasians, plasma levels of ACE relate to the I/D genotype, with the highest levels in DD and lowest levels in II homozygotes [15].

Recently, we have shown that the presence of ACE D allele increased the risk of SSc [16]. The aim of this study was to evaluate the possible macrovascular vessel-wall modifications in SSc and to verify if ACE I/D polymorphism is involved to macrovascular disease.

Patients and methods

The study population included 53 out of 73 consecutive non-hypertensive SSc Italian patients referred to the Section of Rheumatology of the University of Florence and 53 healthy subjects, comparable for age and sex, previously investigated [16]. Fifty-three SSc Italian patients included 47 females and 6 males (median age: 60.4 ± 10.68 yrs; range 40–75 yrs), and the mean disease duration, calculated from the onset of the first non-Raynaud’s symptom, was 9.4 ± 7.8 yrs.

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Fifty-three healthy controls (40 women and 13 men) of the same ethnicity were recruited (median age: 56.3 ± 10.23 yrs; range 40–70 yrs). Exclusion criteria for controls were the anamnestic presence of any vascular disease.

According to Le Roy et al. [17] patients were classified as limited (45 patients) or diffuse SSc (8 patients). Consanguineous subjects were excluded. All subjects signed on informed consent.

The study complies with the Declaration of Helsinki and was approved by the local ethic committee. All subjects signed on informed consent.

**Genetic analysis**

Peripheral venous blood samples were collected from the antecubital vein, with minimal stasis, in Vacutainer tubes containing 0.129 M of sodium citrate, with a final blood/anticoagulant ratio of 9:1. ACE polymorphism were analysed after genomic DNA extraction from peripheral blood leucocytes using a QIAmp Blood Kit (QIAGEN, Hilden, Germany). The ACEI/D polymorphism was genotyped according to Rigat et al. [14]. The detection of the 287 bp I/D polymorphism was performed by polymerase chain reaction (PCR) using primers that span the I/D site and give PCR products of 490 bp for the insertion allele and 190 bp for the deletion allele. We amplified DNA with 5% dimethylsulfoxide in the reaction mixture at an annealing temperature of 67°C to reduce the incidence of mistyping ID as DD. Each DD genotype was subjected to a second PCR amplification without the 5% dimethylsulfoxide at an annealing temperature of 67°C and by using a primer air that recognized the insertion-specific sequence. These modifications were made to reduce underestimation of heterozygotes [16].

**Non-invasive macrovascular assessments**

**Intima–media thickness (IMT).** IMT measurements were performed using an Ultrasound scanner (ATL 3000) with a 7-MHz linear transducer aperture of 38 mm [18]. The ECG signal (lead II) was simultaneously recorded to synchronize the image capture of the top of the R wave to minimize variability during the cardiac cycle. The left and right carotid arteries were scanned at the level of the bifurcation, and images for IMT measurements were recorded from the far wall in the common carotid artery (Fig. 1). The software program gives the average thickness of the IMT. IMT was defined as the distance from the leading edge of the lumen-intima interface to the leading edge of the media-adventitia interface of the far wall (18).

**Fig. 1.** (A–C) IMT measurements at the common carotid artery at the level of the bifurcation. The software program gives the average thickness of the IMT. IMT was defined as the distance from the leading edge of the lumen-intima interface to the leading edge of the media-adventitia interface of the far wall (18).

<table>
<thead>
<tr>
<th>Authors</th>
<th>Disease duration (yrs)</th>
<th>Number of patients</th>
<th>Results</th>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Youssef et al.</td>
<td>&gt;10</td>
<td>4</td>
<td>11% patients with macrovascular disease (upper and lower limbs)</td>
<td>Clinical—physical examination</td>
</tr>
<tr>
<td>Youssef et al.</td>
<td>5</td>
<td>31</td>
<td>58% patients with peripheral vascular disease vs. 9.6% controls</td>
<td>Clinical—Doppler—Angiography</td>
</tr>
<tr>
<td>Veale et al.</td>
<td>5</td>
<td>53</td>
<td>21.7% patients with intermittent claudication vs 4.6% controls</td>
<td>Questionnaire</td>
</tr>
<tr>
<td>Stafford et al.</td>
<td>15</td>
<td>20</td>
<td>Significant thickening of the ulnar arteries in patients vs controls</td>
<td>Doppler</td>
</tr>
<tr>
<td>Ho et al.</td>
<td>4</td>
<td>54</td>
<td>64% patients with carotid artery disease vs 35% controls (P=0.007)</td>
<td>Doppler—ABPI</td>
</tr>
<tr>
<td>Wan et al.</td>
<td>13</td>
<td>119</td>
<td>Not significant reduction of ABPI in patients vs controls</td>
<td>ABPI</td>
</tr>
<tr>
<td>Dick et al.</td>
<td>26</td>
<td>No controls</td>
<td>All selected patients with upper (ulnar and radial artery) and lower limb involvement</td>
<td>Angiography</td>
</tr>
<tr>
<td>Taylor et al.</td>
<td>15</td>
<td>No controls</td>
<td>All selected patients with upper (ulnar and radial artery), lower limb not investigated</td>
<td>Allen test—Angiography</td>
</tr>
<tr>
<td>Bartoli 2006</td>
<td>9.4</td>
<td>53</td>
<td>37.7% patients with carotid disease</td>
<td>Doppler—IMT carotid artery</td>
</tr>
</tbody>
</table>

**TABLE 1. The literature on macrovascular involvement in SSc is presented**
bulb). Normal IMT value is <0.9 mm [20]. Later stages of AS (plaque, stenosis, occlusion) can also be identified by ultrasound imaging either in the absence of or coincident with increasing IMT. Plaque is defined as a focal structure that enroaches into the arterial lumen of at least 0.5 mm or 50% of the surrounding IMT value or demonstrates a thickness of ≥1.5 mm as measured from the media-adventitia interface to the intima-lumen interface [21].

**Ankle-brachial pressure measurements (ABPI).** Carotid duplex scanning (ATL 3000 with a 7.5 Mhz linear probe) was performed by a vascular technician. After a 10 min rest in a comfortably warm room, bilateral ankle and brachial arterial systolic pressures were measured using a portable Sonicaid Doppler probe and Hawkey random zero sphygmonanometer. The WI was calculated as the posterior tibial artery pressure in millimetre of mercury divided by the brachial pressure, and this was done at a later date, during the analysis of the data. The normal WI is 1.0, and any value <1.0 is considered to be abnormal [22] with the severity of the arterial disease being inversely proportional to the WI. We selected a definitive WI cut off point of <0.9 because this criterion has a 95% sensitivity and 100% specificity for detecting arterial disease using angiographically defined disease as the gold standard [23,24] (but also evaluated the presence of milder disease with WI of <1.0 and <0.98). By this criteria 3 out of 56 SSC patients showed a <0.9 WI while all control subjects had a WI >1.0.

**Statistical analysis**

Differences between means were assessed by unpaired t-test. Statistical analysis was performed using the Stata 6.0 software for Windows. Macrovascular disease data and their associations with ACE I/D polymorphism were investigated by Student’s test; P-value <0.05 was taken to be statistically significant. The role of ACE I/D polymorphism as a predisposing factor for SSC was identified by univariate logistic regression analysis. Odds ratio (OR) with 95% confidence interval (CI) was determined.

ACE ID polymorphism genotype distribution and allele frequencies were analysed by using the chi-squared test. The overall genotype distribution was in Hardy–Weinberg equilibrium.

**Results**

ACE I/D polymorphism genotype distribution, allele frequency and the study population characteristics are reported in Table 2. In the study population, the genotype distribution and allele frequency was in Hardy–Weinberg equilibrium. A higher prevalence of ACE D allele was observed in SSC patients in comparison with controls (0.60 vs 0.50). At the univariate analysis, ACE D allele was a predisposing factor to SSC (OR: DD + ID vs II = 2.84; 95% CI 1.06–7.63 P = 0.03). SSC patients (n = 53) were divided according to ACE polymorphism genotypes: 18 patients had ACE DD genotype, 28 had ACE ID and 7 ACE II and the D allele frequency was 0.60.

In SSC, we observed that IMT was significantly higher than in controls (0.85 mm ± 0.03 vs 0.68 mm ± 0.01; P < 0.03). Thirty three patients had normal values of IMT (IMT = 0.74 mm) and 20 patients had IMT above the cut-off value of 0.9 mm (IMT = 1.3 mm). Carotid plaques (defined as focal IMT > 1.5 mm) were present in 12 patients with IMT >0.9 mm, while in the group with normal IMT no plaques were detectable.

In SSC patients, IMT values were significantly (P < 0.04) higher in the allele D carriers (DD homozygotes and ID heterozygotes) (0.89 mm ± 0.03) ACE genotypes patients in comparison to that observed in II homozygotes (0.61 mm ± 0.01) (Fig. 2).

In the group of patients with an IMT >1.0 mm, 17 patients carried the ACE D allele and only 3 patients were homozygotes for the I allele.

The prevalence of ACE D allele was higher in lSSc than in dSSc (0.63 vs 0.43) and the percentage of patients with IMT value >1.0 mm was 30.18% in lSScSc and 37.5% in dSSc.

Of 53 patients 12 had hypercholesterolaemia; the IMT in hypercholesterolaemic patients (0.78 mm) was not significantly different from that found in non-hypercholesterolaemic patients (0.74 mm).

When hypertensive patients were included, no significant difference in IMT values was found between hypertensive and non hypertensive patients (0.85 ± 0.03 vs 1.02 ± 0.03, P < 0.09).

In control subjects, no correlation between IMT and ACE polymorphisms was found.

AS far as APBI was concerned, no significant difference in APBI values between patients (1.018 ± 0.10) and controls (1.091 ± 0.11) was found and no association between APBI and ACE I/D polymorphism genotypes was observed.

**Discussion**

The novel finding of the present study is the relationship between ACE I/D polymorphism and the increase of IMT of the carotid artery in SSC patients. In addition, our results confirmed that patients with SSC have an increased prevalence of macrovascular disease, investigated as carotid artery involvement. These results are in agreement with the previous reports that have highlighted the involvement of the macrovasculature of the upper [25–28] and of the lower limbs [4, 29], as well as of the carotid artery [4] in SSC. There seems to be the coexistence of the extension of the vascular SSC injury to the macrovascular circulation and the accelerated AS in patients with SSC.

The pathogenetic mechanisms involved in the changes of the macrovascular vessel wall in SSC are still unknown. However,

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### Table 2. Main clinical features of the patients and controls are presented

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
</tr>
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<tbody>
<tr>
<td>Number of patients</td>
<td>53</td>
<td>53</td>
</tr>
<tr>
<td>Females/males</td>
<td>47/6</td>
<td>40/13</td>
</tr>
<tr>
<td>Median age</td>
<td>60.4 yrs</td>
<td>56.3 yrs</td>
</tr>
<tr>
<td>DD + ID polymorphism</td>
<td>46/38</td>
<td></td>
</tr>
<tr>
<td>II polymorphism</td>
<td>7/15</td>
<td></td>
</tr>
<tr>
<td>lcSSc/dSSc</td>
<td>45/8</td>
<td></td>
</tr>
<tr>
<td>Smoke</td>
<td>7/5</td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolaemia</td>
<td>16/10</td>
<td></td>
</tr>
<tr>
<td>(cholesterol &gt;220 mg/dl)</td>
<td></td>
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<tr>
<td>Hypertension</td>
<td>20/12</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>1/0</td>
<td></td>
</tr>
<tr>
<td>ACE D allele frequency</td>
<td>0.60</td>
<td>0.50</td>
</tr>
</tbody>
</table>
in SSc both endothelial injury and the migration of smooth muscle cells in the intima might be considered two candidate events contributing to the IMT. Moreover, the procoagulative profile, due to an imbalance of the fibrinolytic system observed in SSc patients [13] and the reduction of AG1-7 of RAS might represent further mechanisms involved in vessel wall remodelling. The similarities between SSc and AS, in the histopathology of the affected vessels and in the pathogenesis, are also observed [1].

Our results show for the first time that the thickness of the carotid intima-media in SSc patients is correlated with ACE DD and ID but not with II genotype. Indeed, our data suggest that SSc itself may not foster the development of macrovascular involvement in those patients that do not carry the DD allele.

Several studies specifically addressed the possible relationship between ACE I/D polymorphism and carotid intima-media thickening in non SSc patients [30, 31]. In the Vobarno population study, ACE D allele was found to be associated with carotid intima-media thickening, but not with carotid atherosclerotic plaques [32]. In a Finnish middle aged population, DD homozygotes had a significantly greater carotid intima-media thickening than subjects carrying II or ID genotype [33], while ACE I/D polymorphism were not associated with carotid IMT in healthy young Finnish adults [34]. In Japanese patients, a correlation has been observed between the D allele and the presence of carotid plaques [35].

In our control subjects no correlation was present between ACE polymorphisms and IMT.

In a recent meta-analysis on ACE polymorphisms and IMT evidence of a positive association between the D allele of the ACE gene and common carotid IMT was shown. A significant positive association was present between the D allele and common carotid IMT and the association was stronger among high-risk populations [36].

In conclusion, our findings confirm an increased prevalence of macrovascular disease in SSc patients and demonstrate that the macrovascular damage affects mainly patients carrying the ACE D allele. This suggests that in SSc the presence of ACE D allele may predispose to the involvement of the macrovascular system.

The clinical relevance of our data require further and larger studies in order to confirm the results and to clarify the mechanisms involved in the acceleration of macrovascular disease in SSc.

References

34. Islam MS, Lehtimaki T, Juonala M et al. Polymorphism of the angiotensin-converting enzyme (ACE) and angiotensinogen (AGT) genes and their associations with blood pressure and carotid artery intima media thickness among healthy Finnish young adults-the Cardiovascular Risk in Young Finns Study. Atherosclerosis 2006;188:316–22.

Rheumatology key message

- Macrovascular involvement is evident in SSc. ACE D allele may predispose to a macrovascular disease in SSc.