Association between systemic lupus erythematosus and insertion/deletion polymorphism of the angiotensin converting enzyme (ACE) gene

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Key words: ACE gene insertion/deletion polymorphism, systemic lupus erythematosus, vasculitis, vascular pathology, visceral damage.

ABSTRACT

Objective
ACE takes part in the renin-angiotensin and kallikrein-kininogen systems by creating angiotensin-II and inactivating bradykinin. ACE gene insertion/deletion polymorphism is associated with the level of circulating enzymes - subjects with the DD genotype have higher levels of circulating ACE than subjects with the II genotype and show an increased tendency towards impaired vascular function and structure. Patients with systemic lupus erythematosus (SLE) suffer from differentially expressed vascular pathology. We attempted to determine whether the type of ACE polymorphism could contribute to this pathology.

Methods
101 SLE patients fulfilling the ACR criteria were investigated. The I/D polymorphism was ascertained by PCR, followed by electrophoresis of the amplified fragments and UV visualization.

Results
The frequency of the D allele was higher in the SLE group (0.623) than in the controls (0.520) ($\chi^2$ test, $p < 0.025$). The distribution of the ACE genotype in SLE group was different from that in the control group (p < 0.05). An association between the DD genotype and visceral damage (p < 0.006) was observed.

Conclusion
Our results suggest that in the multifactorially determined vascular pathology of SLE, changes associated with I/D polymorphism could influence vessel wall inflammation (monocyte adhesion and activation with cytokine release, T-lymphocyte metabolism), a tendency towards vascular impairment (neointimal proliferation, vasospasm, platelet activation, myocyte proliferation) and lead to the subsequent ischemia. The ACE gene could serve as the visceral damage indicator in SLE.

Introduction
The complex nature of systemic lupus erythematosus (SLE) has not yet allowed us to unravel the genetic defects and pathogenic mechanism underlying the disease. SLE is considered to be a composite, multigenic disease involving several overlapping autoimmune responses, each one mediated by a distinct genetic profile (1). Our increasing knowledge of the genetics of the immune response and of the pathogenesis of SLE has been paralleled by a potential association of SLE with genetic markers. The frequencies of certain HLAs encoded for the major histocompatibility complex (MHC) were observed to be increased in SLE (1-3). In addition, some particular gene mutations mapped to the MHC region have been described in association with SLE, e.g. the C4A0 allele (4-6), the TNF alpha gene (7, 8), and the Hsp 70-2 gene (9). However, the MHC region is not the only area containing the genes coding for susceptibility and severity. Associations with IL-6, IL-1RA and other genes have also been studied (4, 10, 11). The ACE gene is located on the long arm of chromosome 17 (12) and shows characteristic insertion/deletion polymorphism based on the presence [insertion (I)] or absence [deletion (D)] of the 287 bp long Alu repeat sequence within intron 16 which results in three possible genotypes (DD and II for homozygotes and ID for heterozygotes) (13). The I/D ACE polymorphism is strongly associated with the level of circulating enzyme (14, 15) and plays a key role in the renin-angiotensin and kallikrein-kininogen systems by activating angiotensin-I into angiotensin-II and inactivating bradykinin. These two peptides have opposite effects on vascular tone, smooth muscle cell proliferation, monocyte adhesion, and platelet adhesion and aggregation, being mediated either directly or via various factors such as endotelin, nitric oxide (NO) and others. These processes have been studied in atherosclerosis, but they might well play a role in the pathogenesis of vasculitis and the vascular changes in SLE and different autoimmune vascular impairments. There has been an interesting report of increased ACE activity and rapid bradykinin degradation in untreated patients with SLE or rheumatoid arthritis (16). Remarkably, the ACE inhibitor captopril has been shown to be associated with reduction in blood pressure and...
improvement in the glomerular filtration rate in SLE patients (17). The aim of our work was to investigate whether the type of ACE I/D polymorphism might have a significant association with SLE. Here we described a genetic association between SLE and the ACE gene.

Patients and methods

Patients

One hundred and one unrelated patients (98 women, 3 men) who met the criteria of the American College of Rheumatology (ACR, formerly the American Rheumatism Association) for SLE (18) were investigated (average age 42.6 yrs., range 18 - 77, mean disease duration 3.5 yrs.). All of them are registered in the Slovak Republic SLE register, which covers the whole country. The patients were examined either during regular outpatient check-ups, or while hospitalized at the Research Institute of Rheumatic Diseases. The approval of the local ethical committee was obtained. One hundred and forty-eight randomly selected healthy controls (101 women, 47 men) from the same area with no prior family or personal history of SLE or any other autoimmune disorders were also studied. Clinical features were defined according to the ACR criteria (18). Pleuritis was defined as a convincing history of pleuritic pain or rub heard by the physician to the pertinent items of the SLICC/ACR Damage Index (19).

Methods

DNA isolation was carried out by phenol-chloroform extraction from blood leukocytes. The ACE gene intron 16 I/D polymorphism was ascertained by PCR (13) employing oligonucleotides (sense 5’ CTTGGAGACCACCTCCCATCTT TT 3’ and antisense 5’ GATGTGGCCATCGCAT TCGTG 3’). Reactions were carried out using 0.25 µg of genomic DNA, 3 mM MgCl2, 50 nM KCl, 10 mM Tris HCl pH 8.4, 0.5 mM of each dNTP, and 1 unit of Taq DNA polymerase in a final volume of 40 µl under the following conditions: 30 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 2 min (Techne DB-2D). The PCR products (190 and 490 bp) were electrophoresed on ethidium-bromide stained 1.5 % agarose gel and visualized under UV light.

Statistical analysis

The allele frequencies of ACE polymorphism were determined by gene counting. Differences in the distribution of the ACE genotypes and alleles were analysed by the two-tailed χ2 test using Yate’s correction where appropriate. For the statistical analysis with correction for the number of statistical comparisons, we used the programme Statgraphics 2.6 (Statistical Graphics Corporation, USA).


Results

The clinical characteristics of our SLE patients are given in Table I. The allele and genotype frequencies obtained in the patients and controls are shown in Table II. There was no significant deviation in the distribution of ACE genotypes from the Hardy-Weinberg equilibrium in either the SLE or the control groups. The frequency of the D allele was found to be significantly higher in the SLE group (0.623) compared to the controls (0.520) (χ2 = 5.303, p < 0.025). The distribution of the ACE genotype in the SLE group was significantly different from that in control group (χ2 = 6.15, p < 0.05). The numbers of SLE patients with the DD genotype subdivided according to different classifying parameters are shown in Table III. Except for visceral damage and...
Dysfunctioning of the endothelium is generally very common in SLE. Angiotensin converting enzyme forms part of the sophisticated regulatory system of endothelial control of vascular tone. ACE I/D polymorphism is strongly associated with the levels of circulating enzyme (20). The DD genotype has a roughly two-fold higher level of circulating ACE than the II genotype (14, 15).

Arteries and arterioles from subjects with the D allele display decreased microvascular sensitivity, decreased endothelial capacity for NO release in response to stimulation, and a higher level of circulating enzyme activity (21). Our results demonstrate an association between the D allele of the ACE gene and SLE (62.3% compared with 52.3% in controls). Statistical analysis further shows an association between the DD genotype and visceral damage, implying that ACE gene polymorphism could serve as a separate visceral damage indicator in SLE.

Human endothelial cells express ACE activity both constitutively and in a regulated manner on the plasma membrane as an ectoenzyme with the catalytic site readily available to blood-borne substrates (22). The product of the enzyme’s activity, angiotensin II, stimulates protein synthesis, myocytal growth, and collagen expression. It also activates endothelial angiotensin II receptors, which subsequently stimulate the production of a very strong contracting factor endothelin and, perhaps, the production of other mediators such as plasminogen activator inhibitor (23, 24). Bradykinin, which is cleaved more rapidly in cases of increased enzyme activity, acts on endothelial B2-kinin receptors and is able to stimulate NO formation by endothelial NO synthase and prostacyclin synthesis (25). NO and prostacyclin inhibit platelet adhesion to the vessel wall. In addition, NO inhibits the adhesion of monocytes, which are important components of the inflamed vessel wall and are capable of releasing growth factors and cytokines. This is consistent with the finding that nitric oxide is an endogenous modulator of leukocyte adhesion (26). Similarly, NO shows a potent inhibitory effect on vascular smooth muscle cell migration and proliferation (27). Modification of the local hormonal equilibrium might result in increased neointimal proliferation, increased cellular matrix formation, and monocyte adhesion, as well as in cell activation with the subsequent release of growth factors and proinflammatory-acting cytokines, platelet aggregation and processes of hemostasis and chronic and acute vasospasm. T lymphocytes have been reported to contain high levels of ACE in their microsomal fraction, where it acts as the major membrane-bound bradykinin-inactivating enzyme. The enzyme level was significantly higher in DD individuals than in the other genotypes (28). Differing ACE activity could influence T lymphocyte metabolism and hypothetically also its functioning, which could play a role in immunologically mediated processes.

Vasculitis is a very common symptom in SLE, either in the form of mucocutaneous vasculitis (skin lesions usually show leukocytoclastic vasculitis) or as a vasculitis associated with visceral organ impairment (retinal, renal, cerebral, gastrointestinal or other), while the small arteries and arterioles are affected most often. As for the pathogenesis of vasculitis, antigen-antibody complexes are formed in antigen excess and are deposited in vessel walls whose permeability have been increased by vasoactive amines such as histamine, bradykinin, and leukotrienes released from platelets or mast cells as a result of IgE-triggered mechanisms. The complex deposition results in the activation of complement, particularly C5a, which is strongly chemotactic for neutrophils. These cells then infiltrate the vessel wall, phagocytose the immune complexes, and release their intracytoplasmatic enzymes, which damage the vessel wall. As the process becomes subacute or chronic, mononuclear cells infiltrate the vessel wall. The common denominator of the resulting syndrome is the reduction of the vessel lumen with ischemic changes in the tissues supplied by the involved vessel (29).

The multi-step process of vasculitis development and vascular pathology in SLE involves many immunological and vascular events and therefore the products of many different genes may be expected to play a role. Here we hypothesize that in the case of SLE, D allele carriers with a proven tendency towards vascular pathology suffer from more severe vascular complications with unfavourable consequences (ischemia) than I allele carriers. In our experience the presence of vasculitis correlates positively with the presence of visceral impairment. However, we cannot exclude the possibility of an effect of ACE on the metabolism of other peptides in view of its broad substrate specificity, nor can we exclude the possibility that a variant of

### Table III. Distribution of DD genotypes in SLE group divided according to the separate classifying parameters with results of χ2 test comparing distribution in subgroups of patients according to the ACE genotype and classifying parameters.

<table>
<thead>
<tr>
<th>Criterion (n = 39)</th>
<th>DD genotype</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthritis</td>
<td>28/11</td>
<td>0.272</td>
</tr>
<tr>
<td>Malar rash</td>
<td>21/18</td>
<td>0.488</td>
</tr>
<tr>
<td>Discoid rash</td>
<td>26/13</td>
<td>0.923</td>
</tr>
<tr>
<td>Pericarditis</td>
<td>4/35</td>
<td>0.586</td>
</tr>
<tr>
<td>Pneumonitis</td>
<td>7/32</td>
<td>0.242</td>
</tr>
<tr>
<td>Pleuritis</td>
<td>10/29</td>
<td>0.504</td>
</tr>
<tr>
<td>CNS involvement</td>
<td>14/25</td>
<td>0.011</td>
</tr>
<tr>
<td>Hypocomplementemia</td>
<td>28/11</td>
<td>0.888</td>
</tr>
<tr>
<td>Anti-dsDNA antibodies</td>
<td>23/16</td>
<td>0.112</td>
</tr>
<tr>
<td>Visceral damage</td>
<td>32/7</td>
<td>0.006</td>
</tr>
<tr>
<td>Renal disorder</td>
<td>20/19</td>
<td>0.449</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>20/19</td>
<td>0.499</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>13/26</td>
<td>0.196</td>
</tr>
</tbody>
</table>

Pres.=Criterion present; Abs. = criterion absent.

### Table IV. Cross-tabulation of ACE genotype by visceral damage.

<table>
<thead>
<tr>
<th>Visceral damage</th>
<th>Visceral damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE ACE</td>
<td>Absolute</td>
</tr>
<tr>
<td>II</td>
<td>8</td>
</tr>
<tr>
<td>ID</td>
<td>21</td>
</tr>
<tr>
<td>DD</td>
<td>7</td>
</tr>
</tbody>
</table>

CNS involvement, there were no clinical symptoms or laboratory findings of the disease that could classify the SLE patients into groups with a statistically significant difference (Table III). The significance of the criterium visceral damage emerged clearly (χ² = 10.235, p < 0.006) (Table IV).

### Discussion

Dysfunctioning of the endothelium is generally very common in SLE. Angiotensin converting enzyme forms part of the sophisticated regulatory system of endothelial control of vascular tone. ACE I/D polymorphism is strongly associated with the levels of circulating enzyme (20). The DD genotype has a roughly two-fold higher level of circulating ACE than the II genotype (14, 15).
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another gene closely linked to the ACE gene caused the observed association. It would be useful to confirm our findings in other populations with different allele and genotype frequencies. Supporting our observations is the recent report of a similar association between the ACE DD genotype and SLE (30). In further experiments we will investigate the putative relationship between CNS involvement and certain forms of ACE I/D polymorphism. In order to demonstrate the reliability of the observed association, this relationship must be confirmed in an extended cohort of patients with this criterion.

Nevertheless, the observed association between ACE I/D polymorphism and visceral damage may have a potential impact on therapy, because nowadays it is possible to pharmacologically inhibit the renin-angiotensin pathway by using ACE inhibitors or angiotensin-II receptor blockers (losartan, valsartan). The beneficial and protective effect of ACE suppression was proven, e.g. in myocardial infarction patients (23) and the effect was evident particularly in D allele carriers - patients suffering from premature myocardial infarction (15). In SLE as well the successful inhibition of ACE activity could result in clinical improvement in patients with visceral organ involvement (17). In rats, cilazapril is able to prevent monocytic accumulation in the subendothelium (26). The antiinflammatory action of ACE inhibitors has been acknowledged and its mechanism partially revealed. It is in part due to a blunting of transcription factor NF-κB activation, which controls a number of genes associated with tissue inflammation (31).

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References