Angiotensin-converting enzyme gene polymorphism in Kuwaiti patients with systemic lupus erythematosus

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Abstract

Objective

To investigate the frequency of angiotensin-converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism genotypes in patients with systemic lupus erythematosus (SLE), and to study the correlation between I/D polymorphism of the ACE gene and the clinical manifestations of SLE, especially vascular involvement, lupus nephritis and disease severity.

Methods

The frequency of ACE gene I/D polymorphism genotypes was determined in 92 patients with SLE from Kuwait, and compared to that in 100 ethnically matched healthy controls using the polymerase chain reaction.

Results

*The distribution of ACE I/D polymorphism and allele frequencies in SLE patients was not significantly different from controls. Further analyses of SLE patients showed that there was a significant association between DD genotype and Raynaud's phenomenon (*p = *0.008, odd ratio = 5.4, 95% confidence interval: 1.6-18.6). However, there was no significant association between the ACE genotype and lupus nephritis or disease severity.*

Conclusion

No difference was found between the distribution of the ACE genotype in SLE patients and the general population in Kuwait. However, the presence of the DD genotype may confer susceptibility to the development of vascular morbidity.

Key words

Angiotensin-converting enzyme, insertion/deletion polymorphism, polymerase chain reaction, systemic lupus erythematosus.

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Introduction

Systemic lupus erythematosus (SLE) is an inflammatory multi-system disease of unknown etiology with immunologic aberrations. It is known to occur in genetically susceptible patients and is influenced by environmental factors (1). It appears that genetic susceptibility to SLE is affected by polygenic inheritance (2). Genetic association with SLE has been most extensively studied in human leukocyte antigen (HLA) class II polymorphism, the C4A null allele, C1q, Fcγ receptors, T cell receptor, IL-1Ra, IL-6, IL-10, bcl-2, Fas ligand, SSA1 gene, and thiopurine S-methyltransferase (TPMT) gene polymorphism (2-4).

The Angiotensin-converting enzyme (ACE) gene is located on the long arm of chromosome 17 (5), and contains an insertion (I) and deletion (D) polymorphism within intron 16 with the presence or absence of a 287 bp repeat sequence. Of the 3 possible genotypes (DD and II homozygotes and ID heterozygotes), carriers of the DD genotype have the highest levels of serum ACE, while those with the II genotype have the lowest serum levels (6).

ACE is expressed in a wide range of tissues including lung, vascular endothelium, kidney, heart, and testes (7). ACE activates Angiotensin I into Angiotensin II, disactivates bradykinin via the kallikrein-kininogen system, and plays a major role in the renninangiotensin system (RAS). The RAS is an important regulator of arterial blood pressure at both the systemic and tissue levels (8). Angiotensin II increases vascular smooth muscle cell contraction and affects smooth muscle proliferation, monocyte adhesion, platelet adhesion and aggregation, being mediated either directly or via various factors, such as endothelin, nitric oxide and other factors.

Sheikh *et al*. (9) reported increased ACE activity in untreated patients with SLE and rheumatoid arthritis. Endothelial dysfunction is not uncommon in SLE, and the DD genotype was reported to be associated with endothelial dysfunction in the normal young population (10). The DD genotype was more prevalent in patients with SLE than in controls (11). However, another recent study reported a significantly lower incidence of the DD genotype in SLE patients with Raynaud's phenomenon compared to those without Raynaud's (12).

Our aims in this study were to investigate the potential significance of ACE gene polymorphism in the incidence of SLE in the Kuwaiti population, and to determine the impact of the ACE genotype on the clinical manifestations of SLE, especially vascular involvement, lupus nephritis, and disease severity.

Patients and methods

Patients

ACE gene I/D polymorphism genotypes were determined in 92 patients with SLE who were being seen on a regular basis in the rheumatic disease clinics of Al-Amiri and Mubarak Al-Kabeer teaching hospitals in Kuwait. All patients fulfilled the American College of Rheumatology (ACR) criteria for the diagnosis of SLE (13). The diagnosis of SLE was made at least 6 months before the initiation of the study. Detailed clinical information was available on all patients, including age, gender, age at disease onset, clinical manifestations, and disease severity. Patients were considered to have 'mild' SLE if they had mucocutaneous, serositis, and/or arthritis manifestations, and 'moderate-severe' SLE if they had renal, neurological, or hematological involvement. Renal disease attributable to SLE was diagnosed if 24-hour urine protein excretion was greater than 500 mg or hematuria (> 5 red blood cells/field) was present. Renal function was defined by the serum creatinine level (normal < 115 umol/L). Hematological abnormalities related to SLE were defined by the presence of one or more of the following: hemolytic anemia with reticulocytosis or leucopenia (< 4,000/mm^3 total on 2 or more occasions), or lymphopenia (< 1,500/ mm^3 on 2 or more occasions), or thrombocytopenia (< 100,000/mm^3 in the absence of offending drugs). Neurologic disorder was defined by the presence of seizures or psychosis in the absence of offending drugs or known metabolic derangements.

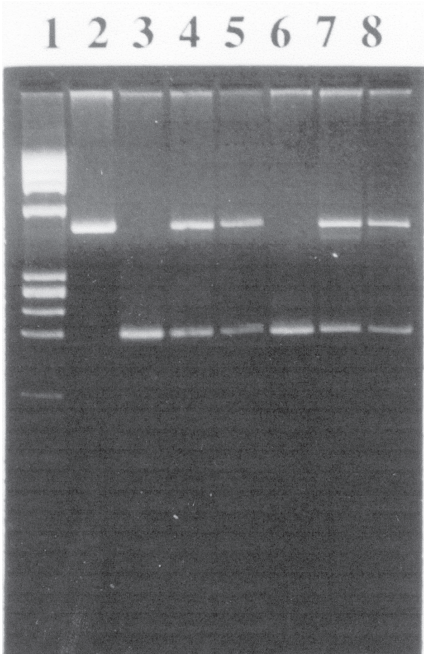

Fig. 1. Detection of ACE gene I/D polymorphism by polymerase chain reaction (PCR). Lane 1: *Hae*III cleaved ϕX174 molecular size markers; lane 2: PCR products from a patient with the II genotype; lanes 3 and 6: PCR products from patients with the DD genotype; lanes 4-5 and 7-8: PCR products from patients with the ID genotype. The products of PCR amplification were analyzed on 2% agarose gel and visualized under UV light following staining with ethidium bromide.

Controls

The frequency of ACE gene I/D polymorphism in SLE patients was compared to that in a group of 100 healthy controls, matched for age and sex with the patients. The control subjects were of similar ethnic background to the patients; all were in normal good health and were being seen at the hospital outpatient clinic for minor illnesses. They did not have a history of autoimmune or rheumatic disorders, or other diseases with a known genetic or hereditary predisposition. A trained rheumatologist examined all the control subjects. A randomization procedure (Pittsburg Genetic Epidemiology Group; M. Trucco, personal communication) was employed for the selection of these controls. None of the patients and controls enrolled in the study were related to each other.

Genotyping

Blood samples were collected from the patients and controls after obtaining their verbal consent. Total genomic DNA was isolated by a standard procedure (14). ACE genotypes were determined by a polymerase chain reaction (PCR) using primers and conditions described previously (13). Reactions were performed with 10 pmol of each primer: sense oligo: 5' CTGGAGACCACTCCCATCCTTTCT 3' and antisense oligo: 5' GATGTGGCCATCACATTCGTCAGAT 3' in a final volume of 50 µl containing 3 mM $MgCl_2$, 50 mM KCl, 10 mM Tris.HCl pH 8.4, 0.1 mg/ml gelatin, 0.5 mM of each dNTP (Cetus), and 2.5 u AmpliTaq DNA polymerase (Cetus). The DNA was amplified for 30 cycles with denaturation at 94ºC for 1 min, annealing at 58ºC for 1 min, and extension at 72ºC for 2 min using a Perkin Elmer Thermal cycler. 0.6% dimethylsulfoxide was routinely added to the PCR mix in order to improve the amplification of the I-allele and thus avoid mistyping it as a D-allele. The PCR products were analyzed on 2% agarose gels after staining with ethidium bromide. In the absence of a 287 bp insertion in intron 16 of the ACE gene, this PCR method resulted in a 190 bp product (D-allele). In the presence of an insertion it produced a 490 bp product (I-allele; Fig. 1). In heterozygous samples, two bands (490 and 190 bp) were detected, along with a third fragment of intermediate size that corresponded to a heteroduplex DNA fragment (15). All analyses were carried out blind and appropriate positive and negative controls were included for each sample run.

Statistical analysis

The significance of the differences in genotype frequencies was evaluated by the chi-square or Fisher's exact tests using Statgraphics software (version 1.1) on an IBM compatible PC. Odds ratio (OR) and 95% confidence interval (CI) values were calculated by comparing the genotype frequency in patients with that in controls. A probability level (p) less than 0.05 was considered significant. The population homogeneity of the study subjects for the ACE gene polymorphism was tested against the Hardy-Weinberg equilibrium ratios by the $\chi 2$ test for genotype frequencies using Gene-POP software (version 3.4) and was found to follow the normal distributions of the Hardy-Weinberg laws.

Results

Ninety-two patients with lupus were enrolled in this study. There were 88 females and 4 males (ratio 22:1). The mean age ± standard deviation (SD) of the patients was 35.6 ± 9.1 years, and their clinical manifestations are shown in Table I. There were 93 females and 7 males (ratio 13:1) in the control group with a mean age (± SD) of 37.6 (± 7.4) years.

Data on the frequencies of ACE gene I/D polymorphism genotypes in Kuwaiti SLE patients and controls are presented

Table I. Baseline characteristics of the systemic lupus erythematosus (SLE) patients enrolled (n = 92).

Gender ratio (female/male)	22:1 (88/4)
Mean age (± S.D), years	35.6 (± 9.1)
Mean age at diagnosis (± SD), years	30.7 (± 8.4)
Median disease duration (range), months	48 (6 – 280)
Clinical manifestations: n (%)	
Malar rash	50 (54.3)
Mouth ulcers	18 (19.6)
Photosensitivity	17 (18.5)
Discoid rash	6 (6.5)
Raynaud's phenomenon	15 (16.3)
Arthritis	88 (95.6)
Serositis	20 (21.7)
Renal involvement	15 (16.3)
Hematological abnormalities	35 (38.0)
Neurological disorders	3 (3.3)
Immunological abnormalities	65 (70.7)
ANA	92 (100)
Hypertension	5 (5.4)

Table II. Angiotensin-converting enzyme (ACE) allele and genotype frequencies in the SLE patients and control subjects.

ACE gene	Patients (n = 92)	Controls (n = 100)	*p* values	OR (95% CI)
Genotype, no. (%)				
DD	37 (40.2)	41 (41)	0.9	0.97 (0.54 - 1.72)
ID	36 (39.1)	45 (45)	0.5	0.79 (0.44 - 1.39)
II	19 (20.6)	14 (14)	0.3	1.60 (0.75 - 3.41)
Allele frequency				
D	0.6	0.64	0.5	0.85 (0.57 - 1.29)
I	0.4	0.36	0.5	1.17 (0.77 - 1.77)

Table III. Distribution of ACE genotype frequencies in SLE patients with and without Raynaud's phenomenon and in controls.

ACE genotype	SLE patients		Controls (n = 100)
	With Raynaud's phenomenon (n = 15)	Without Raynaud's phenomenon (n = 77)	
Genotype, %			
DD	73*	34	41
ID	7	45¶	45
II	20	21	14

*p-value = 0.008 for SLE with Raynaud's phenomenon *vs* SLE without Raynaud's phenomenon and controls (OR = 5.4, 95% CI: 1.6–18.6);
¶p-value = 0.004 for SLE without Raynaud's phenomenon and controls *vs* SLE with Raynaud's phenomenon (OR = 0.09, 95% CI: 0.01–0.69).

Table IV. Distribution of ACE genotype frequencies in SLE patients with and without nephritis and in controls.

ACE genotype	SLE patients*		Controls (n = 100)
	With nephritis (n = 15)	Without nephritis (n = 77)	
Genotype, %			
DD	33	42	41
ID	33	40	45
II	33	21	14

*No significant differences (p > 0.05) between SLE patients with nephritis, patients without nephiritis, and controls for the genotypes.

Table V. Distribution of ACE genotype frequencies in SLE patients with mild and moderate-severe disease.

ACE genotype	SLE patients*		Controls (n = 100)
	Mild (n = 15)	Moderate-severe (n = 77)	
Genotype, %			
DD	40	40	41
ID	42	36	45
II	18	24	14

*No significant differences in genotype between patients with mild or moderate to severe disease and the controls ($p > 0.05$).

in Table II. The frequency of ACE genotypes in the 92 SLE patients and 100 controls was as follows: DD genotypes 40.2% vs. 41%, ID genotypes 39.1% vs. 45%, and II genotypes 20.6% vs. 14%, respectively. There was no significant skewing in the distribution of the ACE genotypes between SLE and controls. The allele frequencies of the D and I alleles were 0.6, 0.4 in patients with SLE and 0.64, 0.36 in controls. There was no skewing in the ACE gene alleles between the two groups. No significant difference ($p > 0.05$) was found in the frequencies of the ACE genotypes and I/D alleles between the patients and controls.

In addition, when a comparison was made between the subgroups of SLE patients – divided into those who had Raynaud's phenomenon and those who did not – a strong correlation was observed for Raynaud's phenomenon. The frequency of genotype DD was found to be significantly higher in patients with Raynaud's phenomenon, as compared to those without and control subjects ($p = 0.008$), whereas the ID genotype was significantly lower ($p = 0.004$) in patients with Raynaud's phenomenon compared to those without or in controls (Table III).

Furthermore, a comparison was made of the homozygous and heterozygous states of the patients. No statistically significant relationship was noted in the ACE allele and genotype frequencies in the subgroups of SLE patients compared to the control group, nor within each subgroup ($p > 0.05$) with regard to nephritis (Table IV) and disease severity (Table V).

Discussion

The present study is the first of its kind to be carried out in patients of Arab origin. It showed no difference in the distribution of ACE genotypes or I/D alleles between lupus patients and the general population in Kuwait. This is in agreement with the studies reported by Molad *et al*. (16), Uhm *et al*. (17), and Lee *et al*. (18). However, our results differ from the findings of previous studies that identified an association of I/D in the ACE genes with SLE. Pullmann *et al*. (19) found a higher fre-

quency of the D allele in SLE patients (62.9%) than in controls (52%) from the Slovak Republic. Interestingly, Tassiulas and co-workers found contrasting results in African-Americans, with the frequency of the D allele being lower in lupus patients (59%) than in controls (72.4%) (20). This agrees with the study by Kaufman *et al*. (7), in that the D allele was higher in African-American controls (68.1%) compared to African-American lupus patients (58.8%), and the difference between these two groups was not significant. The reasons for the divergence in these genotype and I/D allele distributions cannot be explained clearly, but it may be due to racial differences, and unknown genetic or environmental factors.

We report for the first time a significant association of the DD genotype with Raynaud's phenomenon in lupus patients. Raynaud's phenomenon is commonly observed in connective tissue diseases including SLE. Since Raynaud's phenomenon originates from vascular and microvascular lesions, endothelial cell injury or dysfunction has been proposed as a pathogenic mechanism. Evidence for endothelial cell dysfunction in Raynaud's phenomenon includes changes in prostacyclins, thromboxanes (21, 22), and ACE (23). In the normal young population, the DD genotype had an almost twofold higher level of immunoreactive ACE than the II genotype, and the I/D genotype accounted for half the variance in the serum ACE level (6). The DD genotype was also associated with endothelial dysfunction due to a blunting of the stimulated endothelial or donated nitric oxide response (10). These data suggest that the prevalence of Raynaud's phenomenon could be higher in patients with the DD genotype, and this might explain the findings in this study. Several studies have reported an association of the DD genotype with adverse vascular events such as renal artery disease (24), carotid artery disease (25), stroke (26), and coronary artery spasm (27). However, in Israeli and Korean lupus patients, a negative association of the DD genotype with Raynaud's phenomenon and other vascular morbidities was observed (16, 17). The reason for this divergence is not clear. Factors other than ACE polymorphism could affect the presence of Raynaud's phenomenon or serum ACE levels (6).

Our results regarding lupus nephritis are similar to those of Israeli and Korean studies (16, 17), but different from reports on African-American, Chinese, and Japanese cohorts (20, 28, 29) in terms of the lack of association of ACE gene polymorphism with susceptibility to nephritis. These differences could be due to the different genetic backgrounds of these populations.

Our study is the first to demonstrate the lack of association between the ACE genotype and disease severity. However, there is only one recent report in the literature, which supported the correlation of ACE polymorphisms with increasing disease severity in SLE (30). This variance in results may be attributed to the different measures used to assess disease severity and the different genetic backgrounds of the ethnic groups studied.

In summary, I/D polymorphism of the ACE gene did not affect susceptibility to SLE, lupus nephritis, or disease severity in Kuwaiti SLE patients. However, the DD genotype was positively associated with Raynaud's phenomenon in SLE patients.

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