Prevalence of angiotensin-converting enzyme gene insertion-deletion polymorphism in patients with primary knee osteoarthritis

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Abstract

Objective

Angiotensin converting enzyme (ACE) plays an important role in a number of inflammatory and immune related disorders. This study was undertaken to investigate an association between Angiotensin converting enzyme (ACE) gene insertion-deletion (I/D) polymorphism and primary knee osteoarthritis (OA) in Kuwait and to explore a correlation between clinical subgroups of OA and ACE I/D polymorphism genotypes.

Patients and methods

The prevalence of ACE gene I/D polymorphism was determined in 115 patients with primary knee OA and 111 ethnically matched healthy controls by using polymerase chain reaction (PCR) of the genomic DNA. The association of ACE gene I/D polymorphism genotypes was also studied with age of disease onset, function and radiological grading.

Results

No significant difference was detected in the frequency of ACE gene I/D polymorphism genotypes and alleles between knee OA patients and the controls. The frequency of ACE gene polymorphism genotypes was also studied in subgroups on the basis of clinical parameters of age of onset of disease, function and radiological grading and no significant difference was detected between subgroups of OA patients and the controls. This is in sharp contrast to a previous report from Korea in which a significant association has been reported between ACE gene polymorphism and knee OA.

Conclusion

This study did not find an association between ACE gene I/D polymorphism genotypes in Kuwaiti patients with primary knee osteoarthritis and the onset or severity of the disease, which is very different from Korean knee OA patients in which an association has been reported.

Key words

Angiotensin converting enzyme, genotype, polymorphism, osteoarthritis, PCR.

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Introduction

Angiotensin converting enzyme (ACE) plays an important role in a number of inflammatory and immune-related disorders. ACE catalyzes the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor, and by inactivating bradykinin, a vasodialator (1). ACE is present as a membrane-bound enzyme in endothelial cells, in different types of epithelial cells and in synovial fluid. A biallelic polymorphism (insertion-deletion, I/D) was identified in the intron 16 of the ACE gene and it has since been associated with marked differences of serum ACE levels in unrelated healthy individuals (2).

Knee osteoarthritis (OA) is one of the most common musculoskeletal disorders worldwide. It is characterized pathologically by both focal loss of cartilage and marginal and central new bone formation (3). It is estimated that 10% of people over 55 years of age will have disabling knee symptoms (4). The functional disability secondary to knee OA reduces the quality of life and increases the economic burden on the community and hospital. Recent community studies have shown that osteoarthritis is a common problem in Kuwait (5-7). The etiology of knee OA is multi-factorial, including both constitutional factors such as age, sex, obesity, hereditary and local mechanical factors like trauma and occupation. Knee osteoarthritis is recognized to have a genetic component. It is believed to be influenced by multiple genes and several studies have attempted to investigate the association of genes in the prevalence, susceptibility and progression of knee osteoarthritis (8-10). Twin studies, segregation analyses, linkage analyses, and candidate gene association studies have generated important information about inheritance patterns and the genome location of potentially causative mutations, although the results across studies have thus far been inconsistent (10). Knee OA, in particular, has a high prevalence in Asia; recent studies in China show that prevalence of radiographic and symptomatic knee OA among women aged 60 and over were 42.8% and 15.4% respectively (11-12). Epidemiological studies have shown that OA has a strong genetic component, and several susceptibility genes for OA have been reported (13). Kizawa *et al*. (14) first reported a strong association of ASPN gene with knee and hip OA in Japanese patients. However, this association has not been replicated clearly in subsequent studies in European Caucasians (15-17). Jiang *et al*. (12) were however able to replicate the association results in Han Chinese OA patients.

The association of ACE gene I/D polymorphism has also been studied in systemic lupus erythematosus, SLE (18-19) and in systemic sclerosis (20). A role for angiotensin converting enzyme (ACE) gene polymorphism has been suggested in the etiology of knee OA, and a recent study by Hong *et al*. (21) has reported that early onset OA is associated with higher I-allele frequency in Korean patients with knee OA.

In this report, we have studied the prevalence of ACE gene I/D polymorphism in patients with primary knee OA in Kuwait and have investigated its correlation with the age of onset, severity and function of the disease in order to replicate these findings in an ethnically different population/group.

Patients and methods

The study included one hundred and fifteen patients with primary knee OA and 111 healthy control subjects seen in three major teaching hospitals in Kuwait. The inclusion criteria used was: patients with primary knee OA and clinical and radiological evidence of knee OA, with no underlying inflammatory disease or malignancy. All patients had a complete clinical evaluation including demographic characteristics (age, sex), disease onset age, body mass index (BMI), duration of the disease, functional assessment using Lequesne's indices, radiological grading using Kellgren-Lawrence grade (0-4) (22). The control subjects were adult patients with similar ethnic background and were age and sex matched. All had been normal and were seen at the hospital out-patient clinic for minor illnesses. They did not have a history of musculoskeletal system disorders or other diseases with known genetic

or hereditary predisposition. The study has been approved by the ethical committee.

Genotyping

Venous blood samples were collected from patients and controls after obtaining verbal consent. The total genomic DNA was isolated by a standard procedure (23). The genotypes of ACE gene polymorphism were determined by polymerase chain reaction (PCR) using primers and conditions described previously (24). PCR reactions were performed with 10 pmol of each primer: sense oligo: 5'CTGGAGACCACTCCCATCCTTTCT 3' and anti-sense oligo: 5'GATGTGGCCATCACATTCGTCAGAT 3' in a final volume of 50 µl, containing 3 mM $MgCl_2$, 50 mM KCl, 10mM Tris.HCl pH 8.4, 0.1 mg/ml gelatine, 0.5 mM of each dNTP (Applied BioSystems), 2.5 u AmpliTaq DNA polymerase (Applied BioSystems). The amplification of DNA was accomplished in 30 cycles; each cycle consisted of denaturation at 94°C for 1 min, annealing at 58°C for 1 min and extension at 72°C for 2 min using Perkin Elmer Thermal cycler. Dimethylsulphoxide (0.6%) is routinely added to the PCR product to improve the amplification of the I allele and avoid mistyping it as the D allele. The PCR products were analyzed on 2% agarose gels and were visualized after staining with ethidium bromide. In the absence of the 287 bp insertion in the intron 16 of the ACE gene, this PCR method resulted in a 190 bp PCR product (D allele) and in the presence of insertion, 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, which corresponds to a heteroduplex DNA fragment (24).

Statistical analysis

The clinical data of patients with knee OA was analyzed using SPSS (ver. 11) and STATA (SE 8.2, StataCorp, College Station, TX, USA). The significance of distribution of the ACE gene I/D polymorphism was determined and compared using Chi-square test. The *p*-values were considered significant when they were 0.05 or less. The allele and genotype frequencies followed normal Hardy Weinberg distribution. The odds ratio (OR) and 95% confidence intervals (CI) were calculated to quantify the association between ACE genotype I/D polymorphism with disease and age of onset, function and radiological grading.

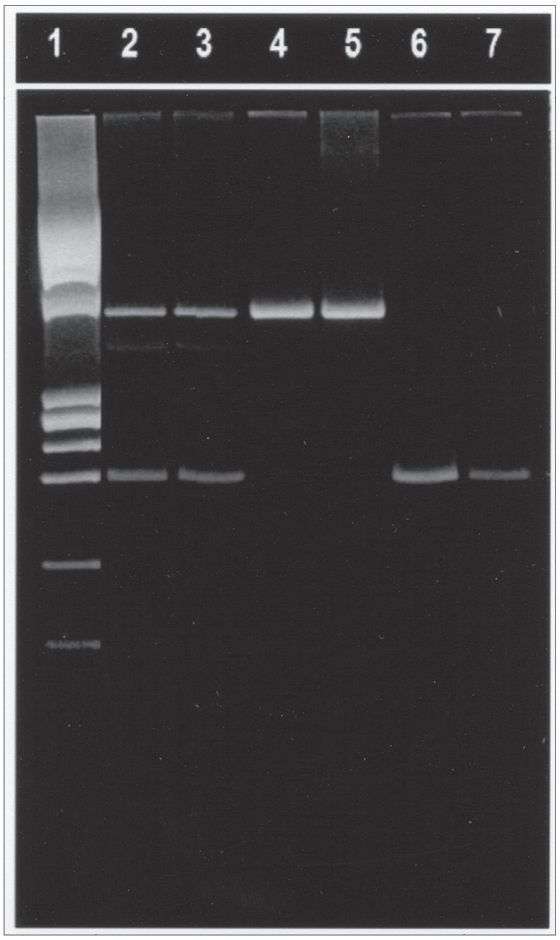

Fig.1. Detection of ACE gene I/D polymorphism by polymerase chain reaction (PCR). Lane 1, *Hae*III cleaved ϕX174 molecular size markers; lanes 2-3, PCR products from patients with ID genotype; lanes 4-5, PCR products from patients with II genotype; lanes 6-7, PCR products from patients with DD genotype.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

Results

This study included 115 patients with primary knee OA. There were 102 females and 13 males, mean age±SD was 57.07±9.15. The study also included 111 healthy controls, 59 females and 52 males with no significant differences between the two groups in age distribution. The characteristics of the 115 patients including age, disease onset age, duration of disease, body mass index (BMI), functional and radiological grading have been presented in Table I. The distribution of ACE genotype and the allele frequencies in patients and controls have been presented in Table II. There was no significant difference in the genotype distribution or the allele frequency between the patient and control groups. Tables III, IV and V show the analysis on association between ACE genotype I/D polymorphism and the clinical subgroups by age of onset of disease (early onset ≤52 years and the late onset >52 years). In

Table I. Clinical characteristics of patients with Knee OA (n=115).

Clinical features	Values
Average age (year, mean ± SD)	57.07 ± 9.15
Male / Female	13 / 102
*BMI (kg/m^2, mean ± SD)	31.71 ± 6.38
Onset age (years)	51.58 ± 7.14
Duration of OA (year ± SD)	5.95 ± 5.04
Kellgren-Lawrence grade	No. of patients
1	17
2	44
3	38
4	16
Function (Lequesne's Indices, mean ± SD)	10.70 ± 4.10

*BMI: body mass index; SD: standard deviation.

Table II. ACE gene polymorphism: genotype and allele frequencies in patients and controls.

ACE gene polymorphism	Patients n=115 (%)	Controls n=111 (%)	*p*-value*
Genotype			0.663
II	23 (20)	19 (17.1)	
ID	22 (19.1)	18 (16.2)	
DD	70 (60.9)	74 (66.7)	
Allele Frequency			0.301
I	68 (29.6)	56 (25.2)	
D	162 (70.4)	166 (74.8)	

*The *p*-values were considered as not significant when they were >0.05.

the subgroups, genotype and allele frequencies showed no significant differences between the patients and controls (Table III).

No significant difference was detected in the ACE genotype and allele frequencies in the subgroups of patients classified on the basis of functional assessment using the Lequesne's functional index and radiological grading using the Kellgren-Lawrence grading (Tables IV and V).

Discussion

In a recent study, Hong *et al*. (21) reported an association of ACE gene I/D polymorphism with primary knee osteoarthritis in a homogeneous Korean population. On the basis of their association data, they suggested ACE gene polymorphism to be a risk factor for early onset, severe form of primary knee OA. We undertook this study in order to replicate these findings in a completely unrelated population (Kuwaiti Arabs). Our results however

Table III. Analysis of clinical subgroups by age of onset of the disease.

	(< 52) Early onset	(> 52) Late onset	OR	*p*-value*	OR	*p*-value**
	n=64	n=51	(95% CI)		(95% CI)	
Clinical subgroup						
K – L grade	2.34±0.96	2.56±0.92				
Functional Index	10.46±4.40	11.01±3.71				
Genotype						
II	13 (20.3)	10 (19.6)		0.925		0.598
ID	11 (17.2)	11 (21.6)		0.553		0.868
DD	40 (62.5)	30 (58.8)	1.17 (0.55-2.46)	0.688	0.83 (0.44-1.58)	0.577
Allele						
I	37 (28.9)	31 (30.4)		0.806	0.83 (0.51-1.35)	0.453
D	91 (71.1)	71 (69.6)	1.07 (0.61-1.89)			

*The chi square test was performed to compare the genotype distribution and allele frequency of ACE gene polymorphism in early onset OA patients with that in late onset OA patients. The *p*-values were calculated at 2 degrees of freedom and were considered as not significant when >0.05
**The chi square test was performed to compare the ACE gene polymorphism in early onset OA patients with that in normal controls. K-L, Kellgren-Lawrence grade; OR: odds ratio; CI: confidence interval. The *p*-values were considered as not significant when they were >0.05

Table IV. Analysis of clinical subgroups by Kellgren-Lawrence grade.

	Severe (Gr 3,4) n=54	Mild (Gr 1,2) n=61	OR (95% CI)	*p*-value*	OR (95% CI)	*p*-value**
Clinical subgroup						
Age of onset	52.8 ±6.7	50.49±7.36				
Functional Index	12.3 ±4.41	9.33±3.27				
Genotype						
II	10 (18.5))	13 (21.3)		0.709		0.824
ID	10 (18.5)	12 (19.7)		0.875		0.712
DD	34 (63)	36 (59)	1.18 (0.56-2.49)	0.665	0.85 (0.43-1.67)	0.639
Allele						
I	30 (27.7)	38 (31.1)		0.576		0.620
D	78 (72.3)	84 (68.9)	1.18 (0.67-2.07)		0.88 (0.52-1.47)	

*The chi square test was performed to compare the genotype distribution and allele frequency of ACE gene polymorphism in severe OA patients with that in mild OA patients. The *p*-values were calculated at 2 degrees of freedom and were considered as not significant when >0.05.
**The chi square test was performed to compare the ACE gene polymorphism in patients with severe OA and normal controls. OR: odds ratio; CI: confidence interval. The *p*-values were considered as not significant when they were >0.05.

Table V. Analysis of clinical subgroups by Lequesne's fractional index.

	Poor index n=53 (%)	Good index n=62 (%)	OR (95% CI)	*p*-value*	OR (95% CI)	*p*-value**
Clinical subgroup						
Age of onset	53.7±6.5	50.49±7.36				
K-L grade	2.7±0.42	2.19±0.90				
Genotype						
II	12 (22.5)	11 (17.7)		0.513		0.398
ID	10 (18.9)	12 (19.4)		0.947		0.673
DD	31 (58.5)	39 (62.9)	0.83 (0.39-1.75)	0.629	0.7 (0.36-1.38)	0.308
Allele						
I	34 (32.1)	34 (27.4)		0.441		0.193
D	72 (67.9)	90 (72.6)	0.80 (0.45-1.41)		0.71 (0.43-1.18)	

*The chi square test was performed to compare the genotype distribution and allele frequency of ACE gene polymorphism in OA patients with poor functional index with that in OA patients who had good functional index. The *p*-values were calculated at 2 degrees of freedom and were considered as not significant when >0.05.

**The chi square test was performed to compare the ACE gene polymorphism in OA patients with poor index that normal controls. K-L, Kellgren-Lawrence grade; OR: odds ratio; CI: confidence interval. The *p*-values were considered as not significant when >0.05

have not found a significant association between ACE gene polymorphism genotypes in OA patients and controls. Also, our data did not find a correlation between the clinical subgroups based on the age of onset and/or severity of the disease. Primary OA is a common and multi-factorial disease with major genetic component(s) and difficult to analyze due to its high frequency in the general population and its wide heterogeneity (25). A wide variation has been reported in the normal distribution of ACE gene alleles in populations of different genetic background. The frequency of the D-allele in normal Caucasians is 50-58%, but 35-39% in normal Chinese (26-28). The genotype frequency of ACE gene polymorphism in normal subjects from Kuwait was found to be 67, 16 and 17% respectively for DD, ID and II genotypes. This is consistent with several previous reports which show a very high incidence of D-allele in our population. In our study, there was no significant difference in the distribution of genotype or allele frequency between control subjects and patients with primary OA. This is exactly what has been observed in the case of Korean study (21). However, the study by Hong *et al*. (18) reported a positive association between I-carriage (non DD genotype) with early onset, functionally poor and radiographically severe primary knee OA. Also, in that report, the allele frequency of I- was found to be significantly higher in early onset OA. However, our results from Kuwaiti Arabs did not find a correlation between any of the genotypes and clinical severity or age of onset of the disease. In our view, the very high incidence of the DD genotype (D-allele) in our Kuwaiti Arab population in general means that most individuals are likely to have higher circulating ACE levels. That is perhaps the reason why we could not find an association between the I-allele and the severity of the disease and the onset age as found in the case of Korean patients. The genetics of OA is complex and is not completely understood, and to assess the validity of reported genetic associations, the best strategy is to reproduce those associations in independent cohorts, preferably focusing on the most clinically relevant phenotypes, which is what we have carried out in this study.

In conclusion, our data from Kuwaiti OA patients does not support the results of Hong *et al*. (21) who have shown that ACE gene polymorphism is a risk factor for primary OA in Korean patients. However, in order to explore this further, more investigations are needed especially in populations with lower general prevalence of the D-allele of ACE gene polymorphism.

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