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Association of angiotensin-converting enzyme (ACE) gene insertion–deletion polymorphism with spondylarthropathies

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Summary

Low back pain (LBP) is a common medical problem. Interaction between genetic and environmental factors predisposes individuals to LBP even at an early age. Inflammatory back pain or spondylarthropathies include ankylosing spondylitis (AS), psoriatic arthritis (PSA), reactive arthritis enteropathic and undifferentiated arthropathies. Angiotensin-converting enzyme (ACE) plays an important role in circulatory homeostasis, physiology of vasculature and inflammation. The insertion–deletion (I/D) polymorphism of the ACE gene has been shown to determine the plasma and tissue levels of ACE especially in the synovial fluid. The aim of this study was to investigate an association between ACE gene I/D polymorphism and inflammatory back pain (spondylarthropathies) secondary to ankylosing spondylitis (AS), psoriatic arthritis, inflammatory bowel disease and undifferentiated spondylarthropathies. The prevalence of ACE gene I/D polymorphism genotypes was determined in 63 patients with inflammatory back pain by polymerase chain reaction (PCR) and compared with that in 111 healthy controls. Of the 63 patients studied, 45 (71.4%) were with AS, 13 (20.6%) were with PSA, 4 (6.3%) were with reactive arthropathy and 1 (1.6%) manifested undifferentiated arthropathy. There were 43 males and 20 females. Mean age of patients was 39.0 ± 11.36 years, age at onset of spondylarthropathy was 27.7 ± 7.49 years and disease duration was 10.3 ± 7.74 months. The controls were selected to match with the patients group in terms of gender ratio, age and ethnicity. The ACE gene polymorphism showed an overall significant difference between patients and controls ($p = 0.050$). When the ID and II genotype frequency was combined and compared with that for DD genotype amongst patient and control groups, a considerably higher incidence was detected for ID and II genotypes than the DD genotype in spondylarthropathy patients compared to that in the controls ($p = 0.036$). This study showed a significant association of the I-allele of ACE gene I/D polymorphism with spondylarthropathy in Kuwaiti Arabs.

Introduction

Low back pain (LBP) is the fifth highest reason for all physician visits [1] and it has been estimated that 50–80% of adults suffer to a significant extent from LBP at some point in life [2, 3]. Recently, we have reported the prevalence of musculoskeletal pain and disability in Kuwait, which was 26.8% and back pain ranks the second 43.8% [4]. We also reported back pain in physical therapist in Kuwait and an interesting observation was that LBP was

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found to be common in adolescents [5–7]. This highlights the role of interaction between genetic and environmental factors in predisposing some individuals to LBP particularly at an early age. Inflammatory back pain or spondylarthropathies include Ankylosing spondylitis (AS), Psoriatic arthritis (PSA), Inflammatory bowel disease associated spondylarthropathies and undifferentiated spondylarthropathies, where genetic factors may contribute to the susceptibility to develop back pain. Recently, the ACE gene insertion/deletion (I/D) polymorphism has been studied in different rheumatic diseases, both in adults and children [8–11]. Plasma ACE measurements have also been used as diagnostic tools. Although plasma ACE concentrations are remarkably stable when measured repeatedly in a normal subject, large inter-individual differences make it difficult to interpret plasma ACE levels in a given patient, as the reference interval for normal value is large [12]. Marker genotypes are useful to identify the alleles exerting a genetic effect on quantitative traits [13]. Circulating ACE activity is a highly heritable quantitative trait [14, 15], and a major QTL (quantitative trait locus) has been shown in linkage and genetic association studies to map within or close to the ACE gene itself [16–18]. Many frequent bi-allelic polymorphisms that are to be associated with high/low values of the quantitative trait have been identified within and flanking the gene [15]. Hubert et al. [19] described a 287 bp insertion in the intron-16 of the ACE gene. The insertion itself corresponded to an *alu* repetitive sequence that resulted in a bi-allelic polymorphism in the ACE gene. Tiret et al. [16] demonstrated that this insertion/deletion (I/D) polymorphism in intron-16 of the ACE gene was strongly associated with marked differences in circulating ACE levels. The marker I-allele appeared always with the major-gene allele- '*s*' characterized by lower ACE levels and the D-allele was associated with markedly higher ACE levels [16]. The function of local rennin–angiotensin system in skeletal muscle and adipose tissue remain largely unknown [20]. Previous studies have shown that level of synovial fluid ACE activity is significantly increased in patients with rheumatoid arthritis [21].

In order to investigate the association between ACE gene I/D polymorphism and spondylarthropathies, we carried out a prospective case–control genetic association study, by screening adult patients with inflammatory back pain for genotypes of the ACE gene I/D polymorphism and compared the incidence of these genotypes with normal control subjects. Therefore, the objective of this study was to investigate whether ACE gene I/D polymorphism confer susceptibility or has a potential role in inflammatory back pain spondylarthropathies secondary to ankylosing spondylitis (AS), psoriatic arthritis, inflammatory bowel disease and undifferentiated spondylarthropathies.

Patients and methods

This study included 63 adult patients with spondylarthropathy and 111 control subjects. ACE gene I/D polymorphism genotypes were determined in patients with inflammatory (spondylarthropathy) LBP who were seen in Rheumatology outpatient clinics in three major hospitals in Kuwait. Patients with underlying inherited/genetic disorders were excluded. Detailed clinical information was available on all patients, including gender, age, age at diagnosis and underlying etiology of all patients. Spondylarthropathies were diagnosed by using European Spondylarthropathy Study Group (ESSG) criteria [22].

The incidence of ACE gene I/D polymorphism was determined in 63 patients and in 111 healthy controls. In the control group, there were 52 males (47%) and 59 females (53%). In selection of the controls, utmost care was exercised to match them with the patients group in terms of gender ratio, age and ethnicity. All the control subjects were adults, 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. Kuwait is a small country of nearly two and a half million inhabitants located in the north western tip of the Arabian Peninsula on the Arabian Gulf coast. Kuwaiti Arabs constitute nearly 45% of the population. The original settlers of Kuwait were immigrants from Najd, an area that now constitutes eastern and central Saudi Arabia. The ethnic origin of Kuwaiti Arabs is quite varied; 50% are of Arab origin, some are Bedouins and nearly 40% are immigrants [23]. The Arabs themselves in most parts of the Gulf countries are the result of an admixture with other populations such as

Persians, Turks, South Asians, Europeans and Africans [23]. The study has been approved by the ethical committee.

Genotyping

Blood samples were collected from patients and controls after obtaining verbal consent. Total genomic DNA was isolated by a standard procedure [24]. ACE gene polymorphism genotypes were determined by polymerase chain reaction (PCR) using primers and conditions described previously [25]. Reactions were performed with 10 pmol of each primer: sensed 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, 10 mM 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 DNA was amplified for 30 cycles with 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. Dimethyl-sulphoxide (0.6%) was added to the PCR mix to improve the amplification of the I-allele. The PCR products were analyzed on 2% agarose gels and were visualized under UV light after staining with ethidium bromide. In the absence of the 287 bp insertion in 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; Figure 1). In heterozygous samples, two bands (490 and 190 bp) were detected along with a third fragment of intermediate size which corresponded to a heteroduplex DNA fragment [25].

Statistical analysis

The significance of the differences in genotype frequencies were evaluated by the Fisher Exact test using SPSS software (ver. 14) on an IBM compatible PC. The *p*-values were considered significant when they were 0.05 or less. The allele and genotype frequencies followed normal Hardy Weinberg distribution.

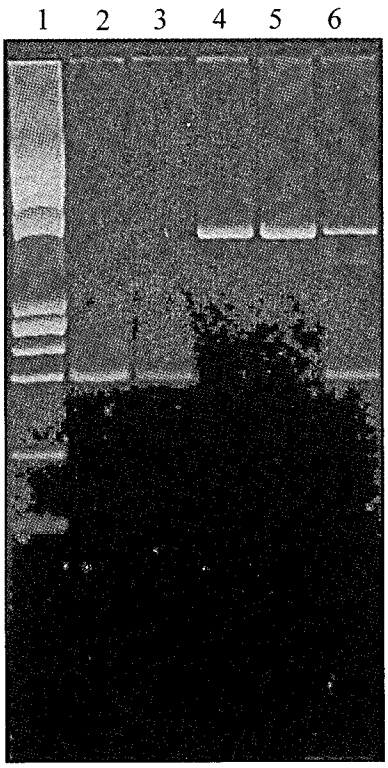

Figure 1. Detection of ACE gene I/D polymorphism by polymerase chain reaction (PCR). Lane 1, *Hae*III cleaved ϕX174 molecular size markers; lanes 2 and 3, PCR products from patients with DD genotype; lanes 4 and 5, PCR products from patients with II genotype; lane 6, PCR products from patients with ID 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 63 patients with spondylarthropathies. There were 43 males and 20 females. Of the 63 patients, 45 (71.4%) were patients with AS, 13 (20.6%) were with PSA, 4 (6.3%) with reactive arthropathy and 1 (1.6%) with undifferentiated arthropathy. The mean age of patients (± SD) was 39.0 ± 11.36 years. The age of onset of arthropathy was 27.7 ± 7.49 years and the disease duration was 10.3 ± 7.74 months (Table 1). In addition to the patients, we studied a group of 111 control subjects which consisted of 52 males and 59 females of similar ethnic background. The incidence of ACE gene polymorphism showed an overall significant difference between patients and controls ($p = 0.050$; Table 2). The incidence of ID genotype was found to be significantly higher in

Table 1. The distribution and characteristics of spondylarthropathy patients in different clinical subgroups (grouped on the basis of associated phenotypes).

	No.	Percentage (%)
Male	43	68.3
Female	20	31.7
AS[a]	45	71.4
PSA[b]	13	20.6
Reactive spondylitis	4	6.3
Undifferentiated	1	1.6
Age (years, mean)	39.0 ± 11.36	
Disease duration (months, mean)	10.3 ± 7.74	
Age of onset (years, mean)	27.7 ± 7.49	

[a]AS, ankylosing spondylitis.
[b]PSA, psoriatic arthritis.

patients than in the controls 30.2% compared to 16.2% respectively (Table 2). The incidence of DD genotype was significantly higher in the controls compared to that in the patients 66.7%, 49.2% respectively (Table 2). The allele frequencies for I- and D-alleles were also compared between the patients and control groups and found to be significantly different ($p = 0.038$; Table 2). The frequency of I-allele was higher in the patient group compared with that in the controls, 35.7% and 25.2% respectively (Table 2). The frequency of ID and II genotypes was combined and compared to the DD frequency between the patient and control groups. This showed a significantly high incidence of ID + II genotypes in the spondylarthropathy patient group compared to that in the controls ($p = 0.036$, Table 2). The ACE genotype and the allele frequency I/D were also studied within the subgroups of spondyloarthropathies (AS, PSA, Reactive spondylitis and undifferentiated arthropathy). There were no significant differences between the subgroups of spondyloarthropathies. Also, no significant difference was detected when the ACE genotype and I/D allele frequency were studied with onset of the disease, HLA B27 (data was available in 52.3% patients) and presence or absence of extraarticular manifestations (data was available in 68.2% patients; Table 3).

Discussion

Low back pain is a pervasive problem that affects two thirds of all adults at some time in their lives. Most often, LBP is benign and self-limited. However, it is sometimes the presenting symptom that manifests itself in association with a number of chronic diseases. Back pain associated with AS, PSA, reactive and undifferentiated spondylarthropathies is referred to as inflammatory or low back pain (LBP). A recent study [26] has described the clinical criteria for differentiating the LBP from mechanical low back pain (MLBP). LBP is a complex disorder which most probably manifests due to an interplay between a variety of genetic and environmental factors. We have undertaken this study to investigate the role of ACE gene

Table 2. The prevalence of ACE gene I/D polymorphism in spondylarthropathy patients and controls.

	Patients ($N = 63$)		Controls ($N = 111$)		p-Value*
	No.	(%)	No.	(%)	
ACE genotype					0.050
DD	31	49.2	74	66.7	
ID	19	30.2	18	16.2	
II	13	20.6	19	17.1	
(ID + II) vs. DD[a]		50.8		33.3	0.036
Allele frequency					0.038
I	45	35.7	56	25.2	
D	81	64.2	166	74.7	

*The p-values were calculated by Fisher Exact test. The test was performed to compare the genotype and allele frequency of ACE gene polymorphism between patients with spondylarthropathy and control groups. The p-values were considered significant when equal to or < 0.050.
[a]The frequency of ID + II genotypes was compared with DD genotype amongst the patients and control groups.

Table 3. The prevalence of ACE gene I/D polymorphism in clinical subgroups of spondylarthropathy patients with different associated phenotypes.

	ACE genotype			*p*-Value	Allele frequency		*p*-Value*
	II	ID	DD		I	D	
	No. (%)	No. (%)	No. (%)		No. (%)	No. (%)	
Diagnosis				0.48			0.215
AS (45)	11 (24.4)	16 (35.6)	18 (40.9)		37 (41.1)	53 (58.9)	
PSA (13)	2 (15.4)	2 (15.4)	9 (69.2)		6 (23.1)	20 (76.9)	
Reactive (4)	0	1 (25.0)	3 (75.0)		2 (25.0)	6 (83.3)	
Undifferentiated(1)	0	0	1 (100)		0	2 (100)	
Onset				0.731			0.443
Before 39 years (29)	7 (24.1)	9 (31.0)	13 (44.8)		23 (19.4)	35 (29.6)	
After 39 years (29)	5 (16.7)	9 (30.0)	16 (53.3)		19 (16.1)	41 (34.7)	
HLA B27				0.713			0.569
Positive (12)	1 (8.3)	3 (25.0)	8 (66.7)		5 (20.8)	19 (79.1)	
Negative (21)	2 (9.5)	8 (38.1)	11 (52.4)		12 (28.5)	30 (71.4)	
Extraarticular				0.717			0.489
Present (20)	4 (20.0)	7 (35.0)	9 (45.0)		15 (37.5)	25 (62.5)	
Absent (23)	3 (13.0)	7 (30.4)	13 (56.5)		13 (28.2)	33 (71.7)	

*The Fisher Exact test was performed to compare the genotype and allele frequency of ACE gene polymorphism with clinical subgroup of spondylarthropathy patients grouped on the basis of associated phenotypes, age of onset, presence/absence of HLA-B27 and extraarticular manifestations. The *p*-values were considered significant when equal to or less than 0.050. AS, ankylosing spondylitis; PSA, psoriatic arthritis. The numbers in parenthesis in the first column (left side) represent the number of patients in each category.

polymorphism in determining the genetic susceptibility to develop LBP. The rennin–angiotensin system plays an important role in maintenance of body fluid and sodium balance and has been implicated in a number of complex disorders [25, 27, 28]. ACE, a component of the rennin–angiotensin system, hydrolyses angiotensin-I to generate the pressor peptide angiotensin-II. ACE is also involved in the kinin–kallikrein system, where it inactivates the vasodilator peptide bradykinin. Angiotensin-II has been shown to be involved in a cascade of events through the angiotensin type-1 receptor and these events include vasoconstriction, proliferation, hypertrophy, matrix deposition, and stimulation of growth factors [29]. It has been shown that angiotensin type-1 receptor expression parallels mesangial cell differentiation from the primitive pericytes. This complements the in vitro observation that angiotensin-induced proliferation and matrix component biosynthesis in fetal human mesangial cells is blocked by co-administration of an angiotensin type-1 receptor antagonist [30]. ACE is produced by endothelium and mononuclear cells of macrophage origin, and ACE activity is significantly increased in patients with inflammatory arthritis compared to subjects with noninflammatory arthritis [31]. It has been postulated that locally generated angiotensin acts on synovial angiotensin receptors to modulate synovial perfusion and growth [32]. The D-allele of ACE gene polymorphism has been associated with higher plasma and tissue levels of ACE [25]. It is plausible that in our spondylarthropathy patients with DD genotype, this higher local concentration of ACE could trigger proliferative actions, e.g. by stimulating inflammatory mediators such as cytokines, which in turn induce arthrogenic changes. In our spondylarthropathy patients with II genotype, lower ACE levels in plasma and/or in the joints possibly increased bradykinin levels to trigger an alternative pathway that caused the inflammatory back pain. It appears from our data that this later mechanism may be more prevalent in our LBP patients. This is further substantiated by a significantly higher incidence of heterozygous genotype (ID) in the patient group compared to the control (Table 2). Our data demonstrates that the possession of an I-allele may constitute a higher risk for

developing spondylarthropathy in Kuwaiti Arabs. This is the first report of an association between ACE gene I/D polymorphism and spondylarthropathies and it would be interesting to compare these findings with data from other populations/ethnic groups when it becomes available.

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