

Angiotensin Converting Enzyme Gene Insertion-Deletion Polymorphism Is Associated with Juvenile Rheumatoid Arthritis

KHALED ALSAEID, M. ZAFARYAB HAIDER, and ELIA M. AYOUB

ABSTRACT. Objective. To investigate the incidence of angiotensin converting enzyme (ACE) gene insertion-deletion (I/D) polymorphism genotypes in children with juvenile rheumatoid arthritis (JRA), a heterogenous chronic disease with autoimmune pathology. ACE gene I/D polymorphism influences the plasma and tissue levels of ACE and has an involvement in inflammatory mechanisms.

Methods. The incidence of ACE gene I/D polymorphism genotypes was determined in 82 children with JRA from Kuwait and compared to that in 48 ethnically matched healthy controls using polymerase chain reaction.

Results. A considerably higher incidence of II genotype was observed in the JRA patients compared to controls ($p < 0.003$). In contrast, no statistically significant difference was detected in the incidence of DD and ID genotypes in JRA patients and controls ($p = 0.276$ and 0.460 , respectively). The incidence of ACE gene polymorphism genotypes was also studied in clinical subclasses of JRA patients and controls. There was no significant difference in the incidence of DD and ID genotypes in either of the 3 JRA subclasses (oligoarticular, polyarticular, and systemic) when compared to controls. However, the incidence of II genotype was found to be significantly higher in all the 3 JRA subclasses compared to controls. The strongest association between II genotype and JRA subclasses was detected in systemic JRA, followed by oligoarticular and polyarticular JRA. This was also reflected in a higher prevalence of I- allele in the systemic JRA cases (13/26, 50%) compared to the D-allele (11/26, 42%). In contrast, D-allele of the ACE gene was more prevalent in oligoarticular and polyarticular JRA cases, than the I-allele (61% and 58%, respectively).

Conclusion. Our data suggest a significant association of the I-allele of the ACE gene I/D polymorphism with the 3 clinical subclasses of JRA in children, and the highest association was observed in systemic JRA cases. (J Rheumatol 2003;30:2705-9)

Key Indexing Terms:

ANGIOTENSIN CONVERTING ENZYME
JUVENILE RHEUMATOID ARTHRITIS

GENOTYPE POLYMORPHISM
POLYMERASE CHAIN REACTION

Juvenile rheumatoid arthritis (JRA) is the most prevalent pediatric rheumatic diagnosis among children around the world. JRA designates a group of diseases that have a common chronic idiopathic inflammation of one or more joints. A variety of evidence points to an autoimmune pathogenesis, including the observed histopathology, the presence of autoantibodies, and the presence of T cell clonality primarily seen in joints¹⁻³. The subtypes of JRA differ in their clinical manifestations, prognosis, and specific auto-

immune characteristics. The clinical features are paralleled by immunogenetic characteristics that have been found to involve primarily, but not solely, the major histocompatibility complex (MHC^{4,5}). Despite the genetic contributions to disease risk observed in many case-control studies, familial JRA is rare. This paradox, i.e., genetic susceptibility with little family history, may be explained by several factors. The low odds ratios of most individual JRA-risk alleles suggest that gene to gene interactions, which are poorly described or understood at present, likely influence whether pathology develops, because no one gene or mutation is sufficient to result in disease but must interact with one or more other genetic factors. The basis for differences between the individual autoimmune arthropathies that constitute JRA is likely to be highly relevant to any understanding of the genetic basis of JRA. While members of families of JRA probands appear to have increased risk of developing the disease, this risk is modest and families with more than one affected member are reportedly rare⁶. Other studies showed higher levels of concordance in the clinical

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subtypes of JRA in affected sibpairs and parent and child pairs⁷. Several studies have described the association between the variation at other genes and JRA⁸. Proinflammatory mediators, e.g., cytokines, play a central role in the pathophysiology of JRA by recruiting and activating immunocompetent cells responsible for the destruction of cartilage, which characterizes JRA. Genetic variations have been reported for a number of molecular mediators of these mechanisms. Angiotensin converting enzyme (ACE) is a critical component of the renin-angiotensin system, and a large body of evidence indicates its proinflammatory role⁹⁻¹⁰. ACE affects various immune phenomena through the renin-angiotensin and kallikrein-kininogen systems by creating angiotensin II and inactivating bradykinin. ACE is also expressed in the synovial membranes from patients with the adult form of RA and a role for this enzyme in the pathophysiology has been suggested¹¹⁻¹³. A polymorphism in the ACE gene (insertion-deletion, I/D) has been shown to influence the circulating and cellular ACE concentration⁹. Its D-allele is associated with a higher risk of developing a number of vascular disorders¹⁴⁻¹⁷.

We determined the incidence of ACE gene I/D polymorphism genotypes in children with well defined clinical subgroups of JRA to investigate the role of polymorphism at this gene with pathogenesis of JRA.

MATERIALS AND METHODS

Patients. ACE gene I/D polymorphism genotypes were determined in 82 children with JRA who were seen regularly in the rheumatology outpatient clinic of the Kuwait University Hospital; diagnosis of JRA was made at least 6 months before the initiation of the study. Detailed clinical information was available on all patients, including gender, age of onset, joints affected, presence of enthesitis, presence of extraarticular manifestations including iridocyclitis, antinuclear antibody (ANA), and rheumatoid factor (RF) test results. The patients were classified according to the American College of Rheumatology 1987 criteria^{18,19}. Inclusion criteria were the presence of oligo- or polyarthritis. Patients with positive RF, enthesitis, or history of psoriasis or inflammatory bowel disease were excluded²⁰. Only patients with oligoarticular, RF negative polyarticular, and systemic onset JRA (Still's disease) were included. All patients were examined at 4 to 6 month intervals by an ophthalmologist for iridocyclitis.

Controls. The incidence of ACE gene I/D polymorphism in children with JRA was compared to that in a group of 48 controls comprising children with similar ethnic background who were seen at the hospital outpatient clinic for minor illnesses. They did not have a history of immune disorders or other diseases with known genetic or hereditary predisposition. A trained pediatric rheumatologist ascertained all controls. A randomization procedure (Pittsburgh Genetic Epidemiology Group; Trucco M, personal communication) was employed for selection of controls.

Laboratory screening. ANA in the serum was detected by immunofluorescence as described²¹; a titer of 1:40 or more was considered positive. RF in the serum was measured by nephelometry (Beckman Array Nephelometer) according to manufacturer's recommendations.

Genotyping. Blood samples were collected from patients and controls after obtaining verbal consent. Total genomic DNA was isolated by a standard procedure²². ACE genotypes were determined by polymerase chain reaction (PCR) using primers and conditions as described²³. Reactions were performed with 10 pmol of each primer: sense oligo: 5' CTGGAGAC-CACTCCCATCCTTCT 3' and anti-sense oligo: 5' GATGTGGCCATCA-

CATTCGTCAGAT 3' in a final volume of 50 μ l, containing 3 mM MgCl₂, 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 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 2 min using a Perkin Elmer Thermal cycler. Dimethylsulfoxide (0.6%) is routinely added to the PCR mix to improve the amplification of the I allele and 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 the 287 bp insertion in the intron 16 of the ACE gene, this PCR method resulted in a 190 bp product (D allele) and in the presence of insertion, produced a 490 bp product (I allele; Figure 1). In heterozygous samples, 2 bands (490 and 190 bp) were detected along with a third fragment of intermediate size which corresponded to a heteroduplex DNA fragment²⁰. The results of PCR analysis were further confirmed by direct DNA sequencing in a selected number of samples from patients with the 3 genotypes. All analyses were carried out double blind, and appropriate positive and negative controls were included for each sample run.

Statistical analysis. The significance of the differences in genotype frequencies were evaluated by Fisher's exact test using Statgraphics soft-

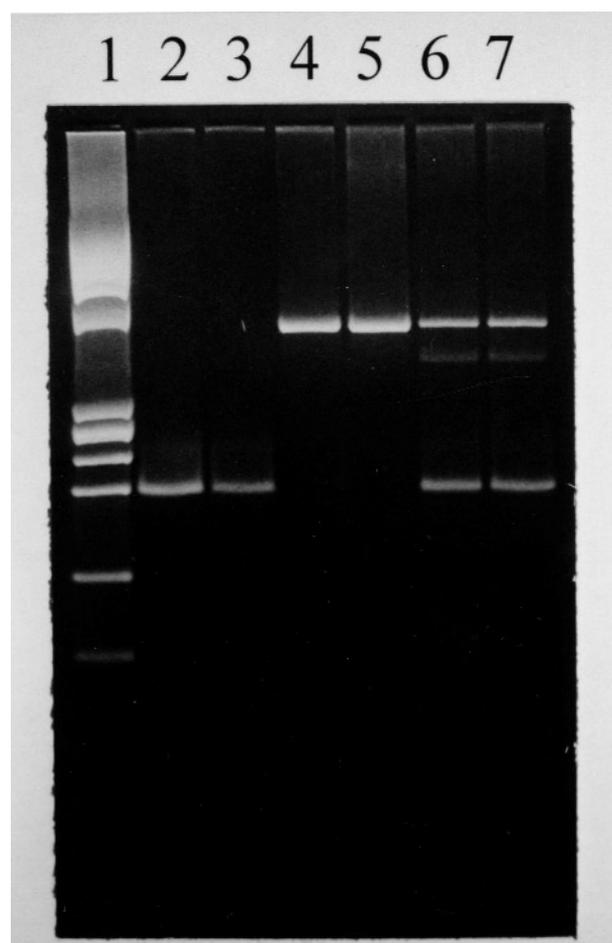


Figure 1. Detection of ACE gene I/D polymorphism by polymerase chain reaction (PCR). Lane 1, HaeIII cleaved ϕ X174 molecular size markers; lanes 2-3: PCR products from patients with DD genotype; lanes 4-5: PCR products from patients with II genotype; lanes 6-7: PCR products from patients with a heterozygous ID genotype. The products of PCR amplification were analyzed on 2% agarose gel and visualized under UV light following staining with ethidium bromide.

ware (version 1.1) on an IBM compatible PC. Relative risk (RR, 95% confidence interval, CI) values were calculated by comparing the genotype frequency in the 3 JRA clinical subclasses to that in the controls.

RESULTS

The patient group consisted of 82 children, of whom 50 had oligoarticular, 19 had rheumatoid negative polyarticular, and 13 had systemic onset JRA. Among the patients, 15 were positive for ANA (18.3%) and 6 patients were diagnosed with iridocyclitis (7%). Data on the incidence of ACE gene I/D polymorphism genotypes in Kuwaiti JRA patients and controls are presented in Table 1. A considerably higher incidence of II genotype was detected in the JRA patients compared to controls ($p < 0.003$; Table 1). In contrast, there was no significant difference in the incidence of DD and ID genotypes in JRA patients compared to controls ($p = 0.276$ and 0.460 , respectively, Table 1). The incidence of ACE gene polymorphism genotypes was also studied in clinical subclasses of Kuwaiti JRA patients and compared to controls. As with the total group, there was no significant difference in the incidence of DD and ID genotypes in any of the 3 JRA subclasses (oligo, poly, and systemic, Table 2). However, the incidence of II genotype was found to be significantly higher in JRA subclasses compared to controls (Table 2). The strongest association between II genotype and JRA subclasses was detected in systemic JRA, followed by oligoarticular, and polyarticular JRA (Table 2). This was also reflected in a higher prevalence of I allele in the systemic JRA cases (13/26, 50%) compared to the D-allele

(11/26, 42%). In contrast, D-allele of the ACE gene was more prevalent in oligoarticular and polyarticular JRA cases than the I-allele (61% and 58%, respectively).

DISCUSSION

Information on the significance of ACE gene polymorphism is available on a few pediatric diseases such as nephrotic syndrome²⁴ and IgA nephropathy²⁵. To our knowledge, this is the first such report on ACE gene polymorphism in JRA patients. Our most important finding is the significantly higher incidence of II genotype detected in JRA patients compared to controls. This association of II genotype was also reflected in all the clinical subclasses of JRA patients. The strongest association between II genotype and disease was detected in systemic JRA cases. A recent study¹⁰ did not find a significant difference between the incidence of ACE gene polymorphism in adults with RA and controls and between severe and non-severe RA. Systemic lupus erythematosus (SLE), another multisystem autoimmune disease, has also been shown to be associated with ACE gene polymorphism. The DD genotype was more prevalent in patients with SLE than in the controls²⁶. Uhm, *et al*²⁷ showed that ACE genotypes were associated with some of the clinical manifestations of SLE but not with others. Another recent study reported a significantly lower incidence of DD genotype in patients with Raynaud's phenomenon compared to those without²⁸.

The renin-angiotensin system plays an important role in

Table 1. The incidence of ACE gene I/D polymorphism genotypes in Kuwaiti JRA patients and controls.

Genotype	JRA Patients, n = 82 (%)	Controls, n = 48 (%)	p (Fisher's Exact)	RR (95% CI)
DD	34 (41)	25 (52)	0.276	0.796 (0.548–1.157)
ID	31 (40)	22 (46)	0.460	0.825 (0.545–1.248)
II	17 (20)	1 (2)	0.003	9.951 (1.366–72.477)

RR: relative risk; CI: confidence interval.

Table 2. The incidence of ACE gene I/D polymorphism genotypes in clinical subclasses of Kuwaiti JRA patients. The frequency of ACE gene polymorphism genotypes was compared in clinical subclasses i.e. oligoarticular, polyarticular, and systemic JRA with that in the controls. The differences in the frequency of DD and ID genotypes between the JRA-subclasses and controls were not statistically significant.

Genotypes	JRA Subclasses		
	Oligo (%)	Poly (%)	Systemic (%)
DD	21/50 (42)	7/19 (36)	3/13 (23)
p*	0.418	0.291	0.115
RR	0.806 (0.528–1.232)	0.707 (0.307–1.353)	0.443 (0.158–1.24)
ID	19/50 (38)	8/19 (42)	5/13 (38)
p*	0.539	1.000	0.758
RR	0.829 (0.519–1.325)	0.919 (0.499–1.692)	0.839 (0.395–1.783)
II	10/50 (20)	3/19 (16)	4/13 (31)
p*	0.008	0.067	0.006
RR (95% CI)	9.600 (1.277–72.195)	7.579 (0.839–68.433)	14.769 (1.801–121.141)

* p values were calculated by Fisher's exact test. RR: relative risk.

maintenance of body fluid and sodium balance and has been implicated in a number of complex disorders^{14-16,29}. ACE, a component of the rennin-angiotensin system, hydrolyzes 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 factors³⁰. 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 antagonist³¹. 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 arthritis¹³. It has been postulated that locally generated angiotensin acts on synovial angiotensin receptors to modulate synovial perfusion and growth³². The D-allele of ACE gene polymorphism has been associated with higher plasma and tissue levels of ACE¹⁴. It is plausible that in JRA patients with DD genotype (mainly oligo and polyarticular JRA cases), 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 JRA patients with II genotype (mainly systemic JRA), lower ACE levels in plasma and/or in the joints may increase bradykinin levels to trigger an alternative pathway that would cause joint inflammation. It appears that this later mechanism may be more functional in systemic JRA cases with II genotype. Our data suggest that the possession of I-allele of the ACE gene polymorphism may constitute a higher risk for developing systemic JRA in children.

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