Pediatr Nephrol (2002) 17:491–495
DOI 10.1007/s00467-002-0902-6

GENETIC RENAL DISEASE

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ACE gene polymorphism and renal scar in children with acute pyelonephritis

Received: 6 August 2001 / Revised: 2 April 2002 / Accepted: 4 April 2002 / Published online: 6 June 2002

Abstract The pathogenesis of renal scarring after acute pyelonephritis (APN) in children is multifactorial. In addition to well-known risk factors (young age, high grade of vesicoureteral reflux, P-fimbriated *Escherichia coli*, and treatment delay), a role for genetic predisposition has been suggested. Since the *ACE* gene deletion polymorphism is a known risk factor for progressive glomerulosclerosis in chronic renal diseases, we have investigated the relationship between the *ACE* genotypes and the development of renal scarring after APN. Fifty-nine children (43 males and 16 females) with APN diagnosed by urine culture and technetium-99m-dimercaptosuccinic acid (^{99}Tc-DMSA) renal scan were studied. *ACE* genotypes were determined as II, ID, and DD using the polymerase chain reaction technique. A follow-up ^{99}Tc-DMSA renal scan was performed to evaluate the development of renal scars 3–6 months after treatment. The distribution of *ACE* genotypes and the allele frequencies were compared in the renal scar-positive (n=39) and -negative group (n=20). *ACE* genotype frequency after stratification by risk factors was also evaluated. The distribution of *ACE* genotypes did not differ between the renal scar-positive (II 25.9%, ID 35.9%, DD 28.2%) and -negative group (II 35.0%, ID 45.0%, DD 20.0%), before and after stratification by each risk factor. *ACE* gene deletion polymorphism did not affect the development of renal scar as an independent variable in children with APN.

Keywords *ACE* gene polymorphism · Acute pyelonephritis · Renal scar

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Introduction

Acute pyelonephritis in children may result in permanent renal scarring that leads to hypertension and chronic renal failure [1]. Multiple risk factors such as younger age, high degree of vesicoureteral reflux (VUR), virulence of the causative organism, and any treatment delay have been shown to be associated with the development of renal scarring [1, 2, 3, 4]. However, the pathogenesis of renal scarring is still controversial and a role for genetic predisposition has been suggested [4, 5].

The angiotensin converting enzyme gene (*ACE*) that regulates the renin-angiotensin-aldosterone system has three genotypes (II, ID, DD), determined by the deletion(D) and insertion(I) of a 287-base pair (bp) segment on intron 16 [6]. The *ACE* gene deletion polymorphism (DD genotype) is associated with the largest amount of angiotensin converting enzyme and angiotensin II, which has hemodynamic, growth, and prosclerotic effects [7]. The *ACE* DD genotype has been suggested as an independent cardiovascular risk factor [8, 9, 10] and also as a predictor of progressive glomerulosclerosis in diabetic nephropathy [11, 12, 13], IgA nephropathy [14, 15, 16], and other chronic renal diseases [17, 18, 19, 20, 21]. In VUR, contradictory results were reported for the *ACE* gene polymorphism and reflux nephropathy [22, 23].

Since the formation of renal scar after acute pyelonephritis is primarily a process of glomerulosclerosis [22], we have prospectively investigated the presumptive role of the *ACE* gene polymorphism in the development of renal scars after acute pyelonephritis in children.

Materials and methods

From February 1999 to February 2002, 59 patients with their first acute pyelonephritis (43 males and 16 females) diagnosed by urine culture and technetium-99m-dimercaptosuccinic acid (^{99m}Tc-DMSA) renal scan were studied at Ewha Woman's University Mokdong Hospital. The *ACE* genotypes were analyzed by polymerase chain reaction (PCR) with informed consent. To extract DNA for *ACE* genotyping, 3 ml of whole blood was centrifuged at

Table 1 Acute pyelonephritis patients with or without renal scar

	Renal scar + *n* (%)	Renal scar – *n* (%)	Total *n* (%)
Male	27 (45.8)	16 (27.1)	43 (72.9)
Female	12 (20.3)	4 (6.8)	16 (27.1)
Total	39 (66.1)	20 (33.9)	59 (100.0)

400 rpm for 20 min and 200 µl of the isolated buffy coat layer was mixed with proteinase K at 70°C for 20 min. An equivalent amount of 100% ethanol was added to precipitate the DNA, which was extracted using a DNA spin column (Quiagen, USA) and was kept frozen at –20°C. PCR was performed with previously published sense primer 5′CTG GAG ACC ACT CCC ATC CTT TCT3′ and antisense primer 5′GAT GTG GCC ATC ACA TTC GTC AGA3′ (Genosys. DNA synthesizer, USA). The 50-µl mixture used for the PCR contained DNA 0.5 µg, 40 pM primer, 0.5 mM dNTP, 3 mM $MgCl_2$, 50 mM KCl, 10 mM TRIS-Cl, pH 8.3, and 1.0 U of Taq polymerase (Boeringer Mannheim, Germany). After 10 min of denaturing at 94°C, 30 cycles of 1 min each at 94°C, 58°C, and 72°C were repeated and the final extension was performed at 72°C for 7 min. Following PCR, the products of amplification were analyzed on a 1% agarose gel and visualized by ethidium bromide staining. The II genotype was expressed as a band in the 490-bp area and the DD genotype in the 190-bp area. The ID genotype showed two separate bands in the 490-bp area and 190-bp area [23]. All DD genotypes were reamplified to rule out the possibility of a shorter D to a longer I with an insertion-specific primer pair 5′TGG GAC CAC AGC GCC CGC CAC TAC3′and 5′TCG CCA GCC CTC CCA TGC CCA TAA 3′.

A follow-up ^{99m}Tc-DMSA renal scan was performed 3–6 months later to evaluate the development of renal scar. Renal scars were classified into four grades according to the classification of Goldraich and Goldraich [24] (type 1 one or two scarred areas, type 2 more than two scars with some areas of normal parenchyma between the scars, type 3 generalized damage to the whole kidney, type 4 end-stage shrunken kidney). Renal scars developed in 38 patients (26 males and 12 females), and did not develop in 20 patients (16 males and 4 females) (Table 1).

ACE genotypes were compared between the renal scar-positive group (n=38) and the renal scar-negative group (n=16). After stratifying the patients by well-known risk factors such as age, grade of VUR, the causative bacteria (*Escherichia coli*), and treatment delay (fever>38.5°C for more than 3 days before treatment), the relationship between the *ACE* genotypes and the development of renal scars was investigated. For statistical comparison, SAS version 6.1 for windows was used for the chi-squared test and Fisher's exact test. A P value of less than 0.05 was considered statistically significant.

Results

The relationship between the *ACE* gene polymorphism and renal scar formation

The distribution of the *ACE* genotypes in the renal scar-positive group (n=39) was II 25.9%, ID 35.9%, and DD 28.2%, which was not different from II 35.0%, ID 45.0%, and DD 20.0% in the renal scar-negative group (n=20) (Fig. 1). The difference was not significant among the four different subgroups by renal scar grading (grade 1 II 20.0%, ID 60.0%, DD 20.0%; grade 2 II 57.1%, DD 42.9%; grade 3 II 28.6%, ID 28.6%, DD 42.9%; grade 4 II 38.5%, ID 53.8%, DD 7.7%) (P>0.05) (Fig. 2).

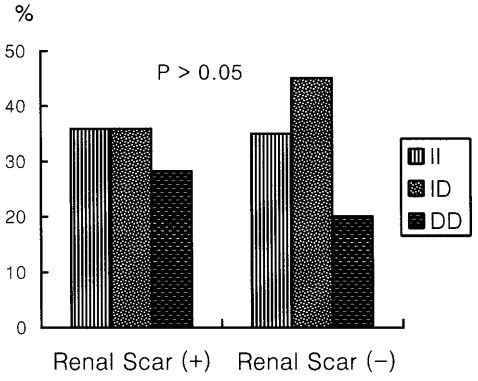

Fig. 1 The distribution of angiotensin converting enzyme (*ACE*) genotypes (II, ID, DD) in the renal scar-positive and -negative groups

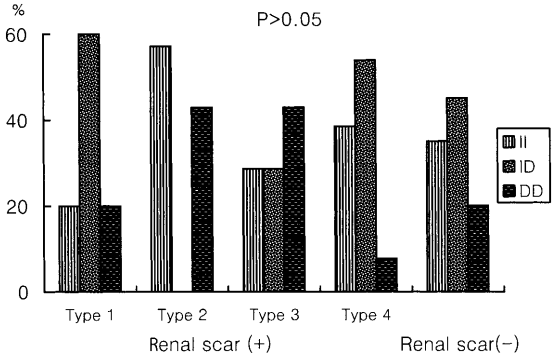

Fig. 2 The distribution of ACE genotypes (II, ID, DD) in the different subgroups by various grades (type 1–4) of renal scars

The relationship between the *ACE* gene polymorphism and renal scarring after stratification by age

In 41 patients less than 1 year of age, the distribution of the *ACE* genotypes of the renal scar-positive group was II 44.0%, ID 28.0%, and DD 28.0%, which was not significantly different from II 36.5%, ID 43.8%, and DD 18.8% in the renal scar-negative group (P>0.05). In 18 patients older than 1 year of age, the distribution of the *ACE* genotypes of the renal scar-positive group was II 21.40%, ID 50.0%, and DD 28.6%. This was not significantly different from II 25.0%, ID 50.0%, and DD 25.0% in the renal scar-negative group (P>0.05) (Fig. 3).

The relationship between the ACE gene polymorphism and renal scarring after stratification by the degree of VUR

In renal units with VUR (n=44), the distribution of the *ACE* genotypes of the renal scar-positive group was II 36.4%, ID 29.5%, and DD 27.5%, which was not different from II 25.0%, ID 25.0%, and DD 50.0% in the renal scar-negative group (P>0.05). In renal units without VUR (n=74), the distribution of the *ACE* genotypes of

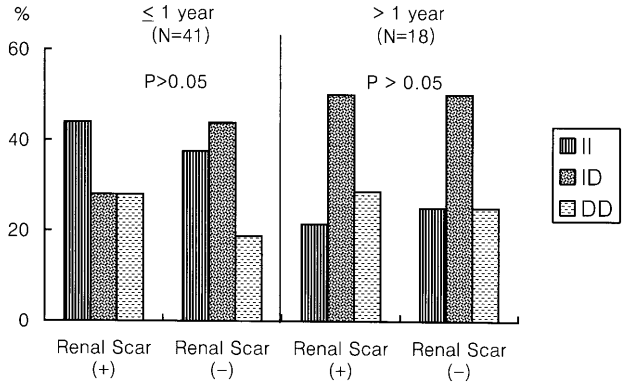

Fig. 3 The distribution of *ACE* genotypes (II, ID, DD) in the renal scar-positive and -negative groups after stratification by age (over 1 year or less)

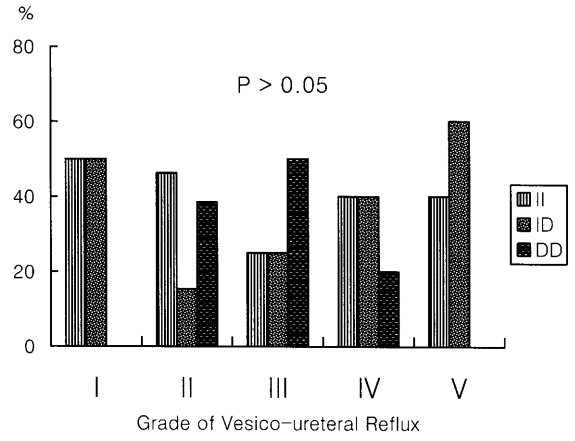

Fig. 5 The distribution of *ACE* genotypes (II, ID, DD) in the different subgroups by grade (I–V) of VUR

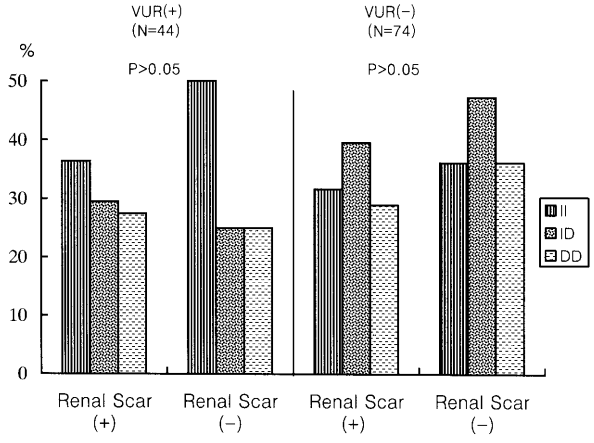

Fig. 4 The distribution of *ACE* genotypes (II, ID, DD) in the renal scar-positive and -negative groups after stratification by vesicoureteral reflux (*VUR*) or not

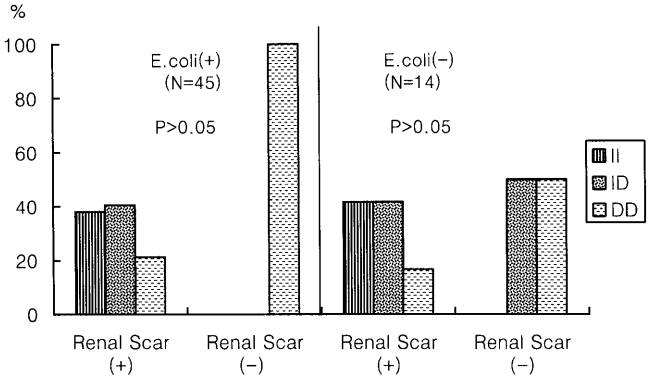

Fig. 6 The distribution of *ACE* genotypes (II, ID, DD) in the renal scar-positive and -negative groups after stratification by the causative organism

the renal scar-positive group was II 31.6%, ID 39.5%, and DD 28.9%. This was not significantly different from II 36.1%, ID 47.2%, and DD 16.7% in the renal scar-negative group (P>0.05) (Fig. 4). No difference was noted among the five different subgroups by the grade of VUR (P>0.05) (Fig. 5).

The relationship between the *ACE* gene polymorphism and renal scarring after stratification by the causative organism

In patients with *E. coli* infection (n=45), the distribution of the *ACE* genotypes of the renal scar-positive group was II 38.1%, ID 40.5%, and DD 21.4%. This was not different from the renal scar-negative group (P>0.05). In patients with non-*E. coli* infections (n=14), the distribution of the *ACE* genotypes of the renal scar-positive group was II 41.7%, ID 41.7%, and DD 16.7. This was not different from II 50.0% and ID 50.0% in the renal scar-negative group (P>0.05) (Fig. 6).

The relationship between the *ACE* gene polymorphism and renal scarring after stratification by the presence of treatment delay

In patients with treatment delay (n=15), the distribution of the *ACE* genotypes of the renal scar-positive group was II 41.7%, ID 25.0%, and DD 33.3%. This was not different from II 66.6%, ID 0.0%, and DD 33.3% in the renal scar-negative group (P>0.05). In patients without treatment delay (n=44), the distribution of the *ACE* genotypes of the renal scar-positive group was II 37.0, ID 37.0%, and DD 25.9%. This was not different from II 29.4%, ID 52.9%, and DD 17.6% in the renal scar-negative group (P>0.05) (Fig. 7).

The frequencies of each genotype

The frequencies of the II, ID, and DD genotypes in the study population were 35.7%, 38.9%, and 25.4%, respectively, which was not significantly different from the

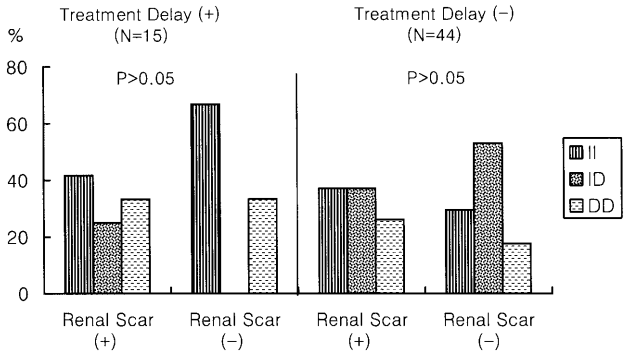

Fig. 7 The distribution of *ACE* genotypes (II, ID, DD) in the renal scar-positive and -negative groups after stratification by treatment delay

Table 2 Allele frequencies in patients with or without renal scar

	Renal scar + *n* (%)	Renal scar – *n* (%)	Total *n* (%)
I allele	42 (53.8)	23 (57.5)	65 (55.1)
D allele	36 (46.2)	17 (42.5)	53 (44.9)
Total	78 (100.0)	40 (100.0)	118 (100.0)

distribution of 37.0%, 40.0%, and 19.0% that was previously reported in 96 healthy Korean controls ($P>0.05$) [23]. The frequency of the I and D alleles was 53.8% and 46.2% in the renal scar-positive group. This was not different from the 57.5% and 42.5% in the renal scar-negative group ($P>0.05$) (Table 2). The genotype frequencies for *ACE* polymorphism were in Hardy-Weinberg equilibrium in both groups.

Discussion

The *ACE* gene, which regulates angiotensin converting enzyme, is located on chromosome 17 across 21 kilobases on 26 exons. The insertion and deletion of the 287-bp DNA fragment determines the genotypes II, ID, and DD [6]. The distribution of the *ACE* genotypes differs between races, and a relatively low percentage of the DD genotype was reported in Koreans and Japanese compared with Caucasians [20]. Individuals with the *ACE* DD genotype have the highest levels of plasma angiotensin converting enzyme and an increased angiotensin II level. The *ACE* II genotype has the lowest level of angiotensin converting enzyme, approximately half of that in the DD genotype. The *ACE* ID genotype has an intermediary level [6]. The intrarenal concentration of angiotensin II in the *ACE* DD genotype is 1,000 times more than that of plasma. It increases the intraglomerular pressure, induces transforming growth factor to exert a prosclerotic activity leading to interstitial proliferation, and activates the plasminogen activator inhibitor (PAI-1) to prevent the degradation of the glomerular interstitium, further aggravating glomerular sclerosis [7]. The *ACE* DD genotype is known as an independent cardiovascular risk factor resulting in hypertension, left ventricular wall hypertrophy, and myocardial infarction [8, 9, 10]. The odds ratio for myocardial infarction was 1.34 in men with the *ACE* DD genotype, even after adjustment for body mass index and serum lipid levels compared with the *ACE* II/ID genotypes [8]. Marian et al. [9] reported a higher prevalence (82.0%) of the *ACE* DD type in those with a family history of sudden cardiac death with hypertrophic cardiomyopathy, irrespective of the severity of hypertension, compared with 34.8% in the normal relatives ($P<0.001$).

The role of the *ACE* gene in chronic renal disease was first suggested in diabetic nephropathy. Controversial results have been reported in diabetic nephropathy and many other different renal diseases. Doria et al. [11] reported that progression in diabetic nephropathy was related to the *ACE* gene polymorphism. In non-insulin dependent diabetes mellitus, the *ACE* DD genotype carried an odds ratio of 2.87 for the development of diabetic nephropathy [12]. But the Diabetic Nephrology Study Group showed no correlation between the *ACE* genotype and progression to nephropathy [13]. Although the *ACE* DD genotype would contribute to the progression of renal disease by increased intraglomerular pressure and matrix proliferation, it may not be the sole factor in the progression to end-stage renal disease.

In IgA nephropathy, the *ACE* DD genotype was more common in end-stage renal disease and the *ACE* II genotype had a more favorable prognosis than the *ACE* DD/ID genotypes [14]. Proteinuria found in patients with the *ACE* II genotype at diagnosis may improve, but this was a less constant feature in patients with the *ACE* ID/DD genotypes. Also, proteinuria significantly decreased in patients with the *ACE* DD genotype after 48 weeks of angiotensin converting enzyme inhibitor treatment, thus predicting the therapeutic efficacy [15]. However, Schmidt et al. [16] reported no difference in *ACE* genotype distribution and allele frequencies between patients with IgA nephropathy and the general population.

In Henoch-Schonlein purpura (HSP) nephritis, the incidence of moderate-to-severe proteinuria at 4–8 years after onset was more than 5 times higher in the *ACE* DD genotype than in the other genotypes [17]. However, Dudley et al. [18] reported no association between disease severity and the *ACE* DD genotype in children with HSP nephritis.

In polycystic kidney disease, cumulative renal survival was significantly less in those with the *ACE* DD genotype than those with the *ACE* ID and the *ACE* II genotypes. The *ACE* DD genotype had an overwhelming frequency of parenchymal damage and an earlier use of angiotensin converting enzyme inhibitors was thought to prevent this damage [19]. The *ACE* DD genotype in children with congenital renal malformations was a significant risk factor for the development of chronic renal failure. This effect was independent of the degree of hypertension and proteinuria [20].

In nephrotic syndrome, those with the *ACE* DD genotype were younger at onset and had more focal sclerosing glomerulosclerosis than minimal change disease compared with the *ACE* II genotype [21].

In a prospective study of 94 children with grade 3–4 VUR, the risk for reflux nephropathy was 4.9-fold in the *ACE* DD genotype that in the *ACE* II genotype [22]. But in a study of 66 children with VUR, the distribution of the *ACE* genotypes in children with reflux nephropathy was II 34.8%, ID 54.5%, and DD 10.6%, was not different from II 38.5%, ID 41.7%, and DD 19.8% in the control group [23].

In our study we have tried to assess the role of *ACE* genetic polymorphism in the development of renal scars after acute pyelonephritis irrespective of VUR. The distribution of the *ACE* genotypes and the allele frequency were not different in the renal scar-positive and -negative groups. The distribution of the *ACE* genotypes was comparable to those from healthy Koreans [23]. Even after stratification by other known risk factors for renal scarring, such as younger age (less than 1 year of age), high degree of VUR, *E. coli*, or treatment delay, there was no difference between the renal scar-positive and -negative group. In conclusion, *ACE* gene deletion polymorphism did not affect the development of renal scarring as an independent variable in children with acute pyelonephritis.

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