Angiotensin converting enzyme (ACE) gene polymorphism in children with primary vesicoureteral reflux

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Objectives: ACE I/D polymorphism was investigated in children with primary vesicoureteral reflux (VUR).

Methods: Sixty-eight children with VUR and 35 healthy controls were enrolled in the study and ACE I/D polymorphism was studied by polymerase chain reaction.

Results: The distribution of ACE I/D polymorphism in patients and controls were DD 36 (53%), ID 25 (37%), ID 7 (10%) and DD 12 (34%), ID 18 (52%), ID 5 (14%) respectively. Patients with VUR were classified into two subgroups according to the presence of renal scars. The age of diagnosis, sum of refluxing kidney ureter unit grades and urinary tract infection frequency were similar in both of the groups. Distribution of ACE I/D polymorphism in patients with scars (n: 34) and without scars (n: 34) were DD 22 (65%), ID 10 (29%), ID 2 (6%) and DD 14 (41%), ID 14 (41%), DD 6 (18%) respectively with a statistically significant difference in between.

Conclusion: We conclude that ACE I/D polymorphism is a predisposing factor in the development of renal scar formation in Turkish patients with primary VUR. The significance of DD polymorphism in renal morbidity should be assessed by national multicenter studies.

Key words: Vesicoureteral reflux, child, ACE polymorphism, renal scar


Introduction

Urinary tract infection (UTI) is the second most common form of bacterial infection in children and if not treated early and properly may lead to hypertension and renal functional loss. Vesicoureteral reflux (VUR) is present in 30-50% of the children with recurrent UTIs.³ 30-50% of patients with VUR may have the risk of reflux nephropathy and 5-10% of these children may progress to end stage renal disease.⁴ The frequency of reflux nephropathy in Turkish children with chronic renal failure is reported to be 32%.⁵ Reflux nephropathy has a progressive course and involves fibrosis and focal segmental sclerosis during the healing of inflammation.⁶ Genes controlling renin angiotensin system play a role in fibrosis.⁷ ACE gene is located on the 17th chromosome. It has 26 exon in a twenty one base place.⁸ The insertion of a 287-base pair fragment in intron 16 of ACE gene is defined as insertion (I) and the absence of a 287-basepair fragment in intron 16 is defined as deletion (D) polymorphism. These polymorphisms are responsible from the serum and tissue ACE levels. Homozygous individuals for the D allele have greater RAS activity and plasma and tissue ACE levels than heterozygotes individuals.

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and II homozygotes. Studies concerning that ACE I and D polymorphisms have roles in Ig A nephropathy, diabetic nephropathy, polycystic kidney disease, VUR and other congenital anomalies had been reported. However, some studies reveal that D allele has no role in renal parenchymal damage. The aim of our study was to investigate the ACE gene I/D polymorphism in children with primary VUR.

Materials and Methods

The study was carried out at Marmara University Medical Faculty, Department of Pediatric Nephrology during July 2000 and July 2003. sixty eight patients (46 girls and 22 boys) who were diagnosed as VUR by voiding cystourethrography (VCUG) and 30 healthy controls (16 girls, 14 boys) were included in this study. Serum creatinine levels, VCUG and DMSA scans were determined. The severity of VUR was graded by VCUG according to International Reflux Classification from grade I to grade V. The sum of the reflux grades of each refluxing kidney ureter unit formed the grade of reflux in each patient. DMSA scan was performed in order to define the presence of renal scars. The presence of focal or generalized decrease in radionuclide uptake and/or renal contour defect was accepted as renal scarring. A second DMSA was repeated after 6 months or 1 year in patients with renal scars.

Genomic DNA was prepared from the EDTA-treated blood. ACE gene I/D polymorphism was studied by polymerase chain reaction (PCR).

Written informed consent was taken from patient families and controls, and the study was approved by the medical faculty ethics committee of our university.

The data was analysed by chi square test with SPSS. A p value lower than 0.05 was considered significant.

Results

The mean age of the patients was 8.6 ± 3.6 years (range 3-16 years), the mean age of VUR diagnosis was 4.6 ± 4 years and the mean age of the controls was 8.2 ± 3.1 years (range 3-14 years). The duration of follow up was 4.7 ± 1.5 years (1.2-7 years) in VUR patients. The mean sum of VUR grades in refluxing kidney ureter units was 3.5 ±1.8 (range 1-8). The mean serum creatinine levels of the patients was 0.45 ± 0.1 mg/dl (range 0.2-0.8 mg/dl). All of the patients had normal glomerular filtration rates and none of them was hypertensive and proteinuric. ACE I/D polymorphism in VUR patients and controls were DD 36 (53%), ID 25 (37%), II 7 (10%) and DD 12 (34%), ID 18 (52%), II 5 (14%) respectively (Table 1).

Patients were classified into two subgroups according to the presence of renal scars. Patients with renal scars were defined as group I (n 34) and patients without scars were defined as group II (n 34). The age at the diagnosis of VUR, the mean VUR grade, frequency of UTI episodes were similar in both of these groups (Table 2). The distribution of ACE gene polymorphism in group I was DD 22 (65%), ID 10 (29%), II 2 (6%) and the distribution of ACE gene polymorphism in group II was DD 14 (41%), ID 14 (41%), II 6 (18%). There was a statistically significant difference between patients with and without scars (Table 3, p< 0.05).

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<tr>
<th>Table 1</th>
<th>ACE polymorphism data of the study population</th>
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<tr>
<td></td>
<td>VUR patients</td>
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<tr>
<td>ACE polymorphism</td>
<td></td>
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<tr>
<td>DD</td>
<td>36 (53%)</td>
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<tr>
<td>ID</td>
<td>25 (37%)</td>
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<tr>
<td>II</td>
<td>7 (10%)</td>
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<tr>
<td>Total</td>
<td>68</td>
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ACE: angiotensin converting enzyme, VUR: vesicoureteral reflux

<table>
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<th>Table 2</th>
<th>The comparison of clinical factors in patients with and without VUR</th>
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<tbody>
<tr>
<td></td>
<td>Patients with scars</td>
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<tr>
<td>Age of diagnosis (year)</td>
<td>4.9 ± 3.4</td>
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<tr>
<td>Sum of refluxing kidney ureter unit</td>
<td>3.8 ± 1.9</td>
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<tr>
<td>Experienced UTI frequency</td>
<td>1.8 ± 1.4</td>
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UTI: urinary tract infection

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<tr>
<th>Table 3</th>
<th>ACE polymorphism in patients with and without renal scars</th>
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<tr>
<td></td>
<td>Patients with renal scar</td>
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<tr>
<td>ACE polymorphism</td>
<td></td>
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<tr>
<td>DD</td>
<td>34</td>
</tr>
<tr>
<td>ID</td>
<td>22 (65%)</td>
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<tr>
<td>II</td>
<td>10 (29%)</td>
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ACE: angiotensin converting enzyme
p: < 0.05, chi square test
Discussion

Recent studies focus on the distribution of ACE gene polymorphism in primary VUR patients and frequently report that DD genotype carries a risk for renal parenchymal damage.20-23 Beyond this, seldom reports reveal that the presence of D allele has no risk on renal scar formation.22 We found that ACE I/D polymorphism is a risk factor for renal scarring in patients with primary VUR.

RAS has major roles on renal development, regeneration and repair processes.24 ACE gene I/D polymorphism regulate plasma ACE levels.24 Individuals with D allele have higher plasma ACE concentrations than individuals with II allele and ID allele.24 DD patients have a more active RAS. Angiotensin II is an octapeptide. Angiotensin II regulates blood pressure, is a potent vasoconstrictor, has dipsogenic action.25 It has strong hemodynamic, prosclerotic and growth promoting effects.26,27 Angiotensin II may induce transforming growth factor (TGF), platelet derived growth factor (PDGF), epidermal growth factor /EGF) and endothelin and results with glomerular hyperthrophy, tubular and mesangial cell proliferation and facilitate mesangial matrix expansion.28-31 This process leading to elevation of collagen and actin synthesis results in glomerulosclerosis and interstitial fibrosis and finally renal scarring.32 Renal fibrosis and renal functional loss are correlated in renal diseases. Age at diagnosis, grade of VUR, delay in treatment of UTI, recurrent UTI are important risk factors for renal scar development.33 Especially children with high grade VUR are at increased risk of renal scar formation.34 Genetic risk factors may also contribute to the development of renal scars. Ozen et al reported that in 94 children with grade III and IV VUR, DD genotype had a 5.4 fold increase for renal scar development.35 Erdogan et al reported that in Izmir area, DD genotype was found to be a significant risk factor in low grade VUR (grade I, grade II, grade III).36 The largest series about this subject is done by Dudley et al in England.22 In the study involving 206 patients with VUR D allele there was no association between the DD polymorphism and presence of renal scarring. Our findings suggest that in 68 Turkish children with VUR DD genotype is a significant risk factor for renal scar development (DD polymorphism in patients with scar was 65%, in patients without scar 41%). DD genotype predicts a worse outcome. The similarity of recurrent UTI attacks and VUR grades in VUR patients with and without scars let us evaluate the risk of the presence of D polymorphism. The differences in results may arise from the different designns of the studies or ethnic variations.

Ethnic differences affect both the population results and the risk of scar development in VUR patients. One of the two different studies of Turkish origin reveal that D allele has a role in high grade VUR, whereas the other display that low grade VUR is associated with D allele.22,23 We included VUR patients with grade I-V VUR in our study and found that DD polymorphism is a significant risk factor in respect to renal scar formation. Dudley’s study showed that DD is not important in the development of scar in English children.22 For our country we need larger series including higher number of patients with multivcenter studies.

In conclusion, ACE I/D polymorphism is a predisposing factor to renal parenchymal damage in Turkish children with primary VUR. The significance of DD polymorphism in renal morbidity should be assessed by national multicenter studies.

References


