RESEARCH BRIEF

Renal Scarring and Osteopontin Gene C/T Polymorphism in Children With Primary Vesicoureteral Reflux

***Hakan Erdogan, #Sevgi Mir, $Afig Berdeli and ±Nejat Aksu**

*From the Departments of *Pediatric Nephrology, Dortcelik Children Hospital, Bursa; #Ege University Medical School, Izmir, $Department of Pediatrics, Laboratory of Molecular Medicine, Ege Univesity Medical School, Izmir, and ±Department of Pediatric Nephrology, Tepecik Training and Research Hospital, Izmir.*

Correspondence to:
Hakan Erdogan, Department of Pediatric Nephrology, Dortcelik Children Hospital, Bursa, Turkey. drerdoganha@yahoo.com
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We examined for *osteopontin* (*OPN*) gene C/T polymorphism in 78 patients (53 girls) with vesicoureteric reflux, with or without renal scarring. The T allele frequency was associated with a significantly increased risk (26.4 fold) of renal scarring.

Key words: *Osteopontin gene, Polymorphism, Reflux nephropathy , Turkey*.

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The development of renal scar is multifactorial in children with vesicoureteral reflux (VUR) [1]. Recent studies have implicated tissue macrophage accumulation in the development of renal scar [2]. The secreted phosphorylated protein osteopontin (OPN) is expressed in a variety of cells including proximal renal tubular cells, and is likely involved in the macrophage infiltration in various models of tubulointerstital disease [3-5].

There are a limited number of clinical studies examining the role of OPN in renal diseases. In these studies, the effects of T/T genotype and T allele freguency on the development of the disease and severity of the disease have generally been studied [6-10]. The aim of this study was to investigate whether *OPN* gene C/T polymorphism plays a role in the development of VUR, the degree of VUR, the development of renal scar and the severity of renal scarring.

Methods

A total of 78 patients (53 girls) with VUR treated in Ege University Medical School were enrolled. The mean age (SD) at diagnosis was 4.1 (3.5) years (range 1 month to 18 years) and the mean (SD) follow-up period was 4.1 (3.0) years. Patients with VUR secondary to neurogenic bladder, lower urinary tract obstruction, duplicated collecting system, ectopic kidney, and additional urinary tract malformation were excluded. The frequency of urinary tract infections (UTI) and the rate of scarring during the follow-up period, as well as the family history of VUR were also recorded.

Voiding cystourethrogram was obtained as soon as the child has completed the course of antibiotic therapy of the UTI. Patients with VUR were divided into two groups; Group 1: low-grade (grades I, II and III), and Group 2: high-grade (grades IV and V). DMSA scintigraphy was performed 4-6 months after acute infection in order to allow acute reversible lesions to resolve. Scar grading was made as type I no more than two scarred areas, type II more than two scarred areas, type III generalized damage, type IV kidney with little or no uptake [13].

In order to determine the *OPN* gene polymorphism heterogeneity, the control group consisted of 61 healthy children (33 boys; mean age: 5.4 ± 3.9 yr) without a history of urological problems or family history of VUR. All of the patients and controls were recruited from the same racial, ethnic, and the same geographical and environment stratification. This study was approved by our Institutional Review Board and informed consent was obtained from parents and control subjects.

Genomic DNA preparation and Genotyping: Genomic DNA from patients and controls was extracted from peripheral blood using a QIAamp Blood kit (Qiagen, Hilden, Germany). DNA concentration was determined by the PicoGreen dsDNA quantitation kit (Molecular

WHAT THIS STUDY ADDS?

- *Increased osteopontin gene T allele frequency might correlate with the increased severity of renal scars.*

Probes Inc., Eugene,OR) and diluted as 100 ng/µL. *OPN* (SPP1) gene polymorphism was genotyped as described previously [10].

Amplification was carried out an a GeneAmp PCR System 9700 (PE Applied Biosystems, Foster City, CA,USA). The PCR product was incubated for 16 hours with 0.5 U *AciI* enzyme (New England Biolabs, Beverly, MA) [10]. Additionally, 20% randomly selected samples for each of the three possible genotypes had formerly been confirmed by sequencing and served as standards.

SPSS version 10.0 for Windows was used for statistical analyses. Differences in categorical variables between groups were tested with Fisher exact and chi-square tests. Differences in continuous variables between groups were tested by the non-parametric Mann-Whitney U test. The compatibility of all the variables to normal distribution was assessed by one-example Kolmogorov-Smirnov test. In the multivariate analysis, results were expressed by the odds ratio (OR) and the 95% confidence interval; P <0.05 was considered significant.

RESULTS

Forty seven patients showed unilateral and 31 patients showed bilateral VUR. Of these, 57 patients (Group 1) had low-grade reflux (grade I in 4, grade II in 19, grade III in 34) and 21 patients (Group 2) had high-grade reflux (grade IV in 16, grade V in 5). Forty patients had no renal scar and 38 showed a renal scar. Renal scar was seen in 21 out of 57 patients (36.8%) in Group 1, whereas 17 out of 21 patients (80.9%) developed renal scar in Group 2. Renal scar type 1 was seen in 15 (19.2%), type II in 10 (12.7%), type III in 7 (9.2%) and type IV in 6 (7.6%) patients. On comparing the *OPN* gene C/T polymorphism between the patient and control groups, T allele frequency was found to be higher in control as compared to the patient group (0.38 *vs* 0.28) (P>0.05). Similarly, T allele frequency was not statistically different between patients with and without renal scar (0.34 *vs* 0.23) and patients with low- and high-grade reflux (0.25 *vs* 0.40).

In one-way analysis, reflux grade was found to be the only risk factor for the development of renal scar (P<0.01, OR 7.2, 95% CI 2.16 , 24.55). Other risk factors such as age, sex, bilateral reflux, family history, frequency of UTI and T allele frequency had no effect on renal scarring. On multivariate analysis, reflux grade was also the only factor renal scarring (P <0.01, OR 4.83, 95% CI 2.14 - 10.91).The T allele frequency correlated positively with scar grading (0.20 in type I, 0.25 in type II, 0.50 in type III, 0.67 in type IV) (P <0.05). The T allele frequency was 0.22 in type I and type II (n=25) and 0.58 in type III and type IV type IV renal scar (n=13). This difference was statistically significant (P <0.05). T allele frequency was not statistically different between patients with no renal scars and type I plus type II group (0.23 vs 0.22) (P >0.05).

T allele frequency and reflux grade increased the development of scarring 4-fold and 8.5-fold, respectively. Age, sex, bilateral reflux, family history, and frequency of UTI were found to be insignificant risk factors on scar severity score in VUR cases (P > 0.05). On multivariate analysis, T allele frequency was the only factor statistically significantly associated with the susceptibility to scar severity (P <0.01, OR 26.4, 95% CI 1.71, 40.8), whereas other factors were insignificant (P >0.05).

DISCUSSION

In the present study, we investigated *OPN* gene C/T polymorphism (on exon 7, position 9250) in children with VUR. Although statistically insignificant, T allele frequency was found to be higher in control group as compared to patient group, suggesting that T allele frequency plays no significant role in the development of VUR. Similarly, no statistical difference was observed between low- and high-grade VUR cases, indicating that the presence of TT genotype or the high T allele frequency does not play a role in the degree of reflux. To our knowledge, this is the first study focusing on the T allele frequency and VUR in children from same ethnicity.

Although the T allele frequency tended to be higher in patients with renal scar, this did not reach statistical significance. Additionally, univariate and multivariate analyses also showed that the T allele frequency had no effect on the development of renal scars. Increased T allele frequency was progressively associated with the increased scar grading. Besides this, the T allele frequency increased the risk of the development of renal scar 4-fold and 26.4-fold, respectively.

The mechanism by which the molecular change induces renal fibrosis is not clearly known [12]. While the present study, does not prove that OPN gene

polymorphism is the sole factor associated either with development of scars or their severity, further studies are necessary.

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