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Association of angiotensin converting enzyme and angiotensin type 2 receptor gene polymorphisms with renal damage in posterior urethral valves

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Abstract *Objective:* To examine the association with renal damage in patients with posterior urethral valves (PUV) of two renin–angiotensin system gene polymorphisms: angiotensin converting enzyme insertion/deletion (ACE I/D) and angiotensin type 2 receptor (AT2R A1332G),
Patients and methods: In 120 patients with PUV, after stabilization, transurethral fulguration or a Blocksom vesicostomy was performed. Records were reviewed for age at diagnosis, biochemical renal function at diagnosis, results of urine cultures, voiding cystourethrograms, radiologic, sonographic and nuclear medicine scan findings, and follow-up data. ACE I/D genotypes were determined by the polymerase chain reaction using allele specific primers.
Results: The frequency of the ACE DD genotype was significantly higher in patients with chronic kidney disease ($P = 0.02$) and renal scarring ($P = 0.05$). These genotypes were also associated with a statistically higher incidence of vesicoureteral reflux, diurnal incontinence, proteinuria and hypertension. A significantly higher frequency of the AT2R GG genotype was found in PUV patients as compared to healthy unrelated control subjects ($P = 0.001$), and in PUV patients with scarring ($P = 0.02$).
Conclusion: The ACE DD and AT2R GG genotypes are associated with chronic kidney disease and scarring in PUV patients. The GG genotype incidence is higher among PUV patients compared to the control population, and further studies in this area may help understanding of the genetic basis of PUV.
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Introduction

Patients with PUV are at significant risk of progression to end-stage renal disease (25%—40%) despite early correction of the obstruction [1]. The search to improve their long-term renal function has steadily increased the quality of management and treatment [2]. Recently, the renin—angiotensin system (RAS) has been shown to be important in the progression of experimental and human glomerulopathies [3]. Experimental studies have indicated that derangement of the RAS during fetal life may be associated with renal maldevelopment [4]. Angiotensin converting enzyme (ACE) is one of the most intensely studied candidate genes in human renal malformations [5,6]. An insertion/deletion polymorphism in this enzyme (ACE I/D polymorphism) has been shown to affect the activity of the RAS. The DD genotype has been suggested as an independent risk factor for renal parenchymal damage in patients with congenital urologic abnormalities [7—10].

The angiotensin type 2 receptor (AT2R) is also considered to be associated with the development of fetal organs, since AT2R is expressed abundantly in fetal tissues [11,12]. Abnormalities in AT2R expression hinder interaction between the ureteric bud and metanephric blastema, and hamper normal development, resulting in congenital anomalies of the kidney and urinary tract (CAKUT). It has been reported that A to G transition at the −1332 position in AT2R (AT2R A1332G) is associated with abnormal splicing, producing shorter length mRNA and thereby affecting AT2R activity. The AT2R A1332G polymorphism has been inconsistently shown to be associated with CAKUT in Caucasians [11,13] and Japanese subjects [14]. However, these studies combined a heterogeneous group of patients with CAKUT, and hence information on the influence of these genotypes on PUV is lacking. In the present study, we examined the association of ACE I/D and AT2R A1332G polymorphisms with renal damage in a large cohort of PUV patients treated by a single surgeon from a tertiary referral centre in India. The aim was to find out whether the RAS genes affected long-term kidney function independently as suggested by some workers previously.

Patients and methods

The principal investigator treated 265 PUV patients from 1992 to 2008. Of these, 30 expired during follow up and 115 were lost to follow up. The past medical records of 120 patients with PUV who were on regular follow up and willing to participate in the study were included. The records of these children were reviewed for age at diagnosis, blood urea nitrogen, serum creatinine, urine cultures, occurrence of breakthrough UTIs, VCUG, sonographic and nuclear medicine scan findings at presentation and follow up, and details of surgical interventions. Micturating cystourethrogram, ultrasound and follow-up radiological studies were reviewed to confirm the diagnosis of valves, status of reflux and follow-up status of the kidneys. The mode of initial treatment and follow up is described in our earlier publications [15,16]. The initial treatment consisted of valve ablation in 90 patients and diversion in 30 patients. The time of closure vesicostomy was 1 year in two patients and 2 years in the rest. Reflux was graded according to the International Reflux Classification [17]. A check cystoscopy was done 6 months after fulguration. At follow-up visits blood chemistry studies, acid base status, urine culture, blood pressure, 24-h urinary protein test and renal scans were monitored. Follow-up 99mTc-DTPA, dynamic renal scintigraphy was performed to monitor renal functions, renal scarring and drainage for the purpose of the study. Scans were reviewed by a consultant for the purpose of the study and he was blinded to the genotypes of the patients. The original scans and follow-up kidney functions on DTPA scans were reported as poor if there was impaired uptake of the radiotracer with raised background activity and flattened time activity curves. DMSA renal scintigraphy was used to study the presence or absence of renal scars. Breach in the cortical outline with no/poor tracer uptake was considered to be suggestive of cortical scars. GFR was calculated from the plasma creatinine concentration and the child's length according to the Schwartz formula [18]. A GFR value of less than 80 ml/min per 1.73 m^2 was considered as reduced function [19]. Staging of chronic kidney disease (CKD) followed the National Kidney Foundation guidelines [20]. Blood pressure was defined as per the criteria addressed by the Task Force on Blood Pressure control in children [21]. Proteinuria of >100 mg/m^2/24 h was considered significant. Healthy children who presented for hospital consultation and underwent blood sampling for other investigations ($n = 96$), with no clinical signs of renal disease and no family history of renal disease, were recruited, after consent was appropriately obtained, as controls for the genotyping studies.

Venous blood (2 ml) was collected in sterile vials containing ethylene diamine tetraacetic acid. DNA was extracted by the phenol chloroform method and was stored at −20 °C.

ACE I/D genotypes were determined by the polymerase chain reaction using allele specific primers method as reported earlier [22]. The sense oligonucleotide primer was 5′-CTG GAG ACC ACT CCC ATC CTT TCT-3′ and the antisense primer was 5′-GAT GTG GCC ATC ACA TTC GTC AGA-3′. Reactions were performed in a final total volume of 50 μl containing 10 pmol of primer, 5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris—HCl, pH 8.4, 0.1 mg/ml gelatin, 0.5 mm of each dNTP and 1 unit of TaQ polymerase. DNA was visualized by ethidium bromide staining [23].

AT2R A1332 genotypes were determined by the PCR-RFLP method [12]. The following primers were used:

5′-GGA AAG TAG AAC AATA CAT TAA ATG-3′ and

5′- CCT GTA AGA GAA ACA GCA GCT AAA GAA -3′. PCR products were digested with the restriction enzyme EcoRI. The A → G transition yielded an EcoRI digestion site which provided RFLP.

Statistical analysis

The data were analyzed using SPSS for windows 11.0 software. Data were expressed as mean plus or minus standard deviation (SD). Statistical comparisons between group means were done by non-parametric test (Mann—Whitney U-test). The genotypic distributions and allelic frequencies were compared between groups by the Pearson chi-square test. Multivariate logistic analysis risk factors, such as UTI,

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