Renal Failure

ISSN: 0886-022X (Print) 1525-6049 (Online) Journal homepage: https://www.tandfonline.com/loi/irnf20

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To cite this article: Ümit Sızmaz Çelik, Aytül Noyan, Aysun K. Bayazıt, Mithat Büyükçelik, Hasan Dursun, Ali Anarat, Gülen Attila & Selçuk Matyar (2006) ACE Gene Polymorphism in Turkish Children with Nephrotic Syndrome, Renal Failure, 28:5, 401-403, DOI: 10.1080/08860220600599084

To link to this article: https://doi.org/10.1080/08860220600599084

Published online: 07 Jul 2009.

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Renal Failure, 28:401–403, 2006
Copyright © Informa Healthcare
ISSN: 0886-022X print / 1525-6049 online
DOI: 10.1080/08860220600599084

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CLINICAL STUDY

ACE Gene Polymorphism in Turkish Children with Nephrotic Syndrome

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Since 1990, the role of angiotensin converting enzyme (ACE) gene polymorphism in various renal and cardiac diseases is still debated. This study comprised 71 pediatric patients with nephrotic syndrome, 47 males (66%) and 24 females (34%) with a mean age of 57.4 ± 37.6 months, and a control group of 83 healthy males (59%) and 57 healthy females (41%) with a mean age of 505 ± 160.5 months. The distribution of the ACE genotype in the control group was II, 11%; ID, 53%; and DD, 36%, and the nephrotic syndrome was II, 4%; ID, 78%; and DD, 18%. Angiotensin-converting enzyme genotypes were significantly different between patients and control groups ($p<0.05$). The study groups consisted of 52 (73%) with steroid-sensitive nephrotic syndrome (SNSS) and 19 (27%) with steroid-resistant nephrotic syndrome (SRNS). The distribution of the ACE genotype was II, 6%; ID, 75%; and DD, 19% in the SSNS population and ID, 84% and DD, 16% in the SRNS population. No statistically significant difference was found between steroid sensitivity and ACE genotypes ($p=0.5$). The results show that ACE I/D polymorphism does not contribute to the steroid resistance, even though this study indicates that the presence of the I/D genotype has a much higher risk—approximately 2.8 times—of having nephrotic syndrome. Further studies with a larger number of patients are needed.

Keywords nephrotic syndrome, childhood, ACE gene polymorphism

This study was supported by the Cukurova University Research Foundation (Nr. 2002 LTP-48). The authors are grateful for the statistical assistance of Dr. Gülşah Seydaoğlu. Also they would like to thank to Halil Gülsev for his help with the laboratory procedures and PD nurse Gülşen Kaygusuz and Dr. Banu Kılınç for their assistance.

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INTRODUCTION

Nephrotic syndrome is neither a single disease nor a heterogenous group of related diseases. Rather, it is a clinical state characterized by heavy proteinuria and hypoalbuminemia, and it is often associated with edema, hypercholesterolemia, and hyperlipidemia.[1] Pediatric nephrologists wonder why some patients have good response and, others do not, and it is difficult to predict at onset the clinical course in terms of steroid responsiveness or resistance. Therefore, with the development of genetics, the role of genes on the response to the treatment was studied intensively in patients with nephrotic syndrome.

Rigat et al. described an insertion/deletion polymorphism (I/D) of the angiotensin-converting enzyme (ACE) gene that accounted for 40% of the interindividual variation in serum and cardiac ACE activity in 1990.[2] ACE is a zinc metallopeptidase that not only converts angiotensin I to angiotensin II but also degrades bradykinin, which regulates vascular tones and cardiac functions. ACE levels are highest in individuals who are homozygous for D allele, lowest in those homozygous for the I allele, and intermediate in I/D heterozygous individuals.[2] Patients who had higher ACE levels were exposed by the risk of angiotensin II (Ang II). Ang II has an important role in renal disease. Its damaging effects are evident in glomerulosclerosis, tubulointerstitial nephritis, and vascular sclerosis. Since 1990, the ACE D allele has been associated with a number of disease states for which activation of renin angiotensin aldosterone system (RAS) has been implicated in playing a role, including acute myocardial infarction, left ventricular hypertrophy and progressive diabetic nephropathy, vesicoureteral reflux, and nephrotic syndrome.[3–8]

The purpose of this study was to examine the ACE I/D genotype distribution in patients with nephrotic syndrome. The authors also evaluated the distribution of

ACE genotypes in patients who were steroid responsive and nonresponsive.

PATIENTS AND METHODS

This study was conducted in the Cukurova University Faculty of Medicine Department of Pediatric Nephrology. The study was approved by the ethical committee of the Cukurova University Faculty of Medicine, and a written informed consent was obtained from the parents. Seventy-one patients with NS and 140 healthy controls were enrolled to the study. The patient group consisted of 47 males (66%) and 24 females (34%) with a mean age of 57.4 ± 37.6 months. The control group consisted of 140 unrelated, healthy, adult volunteers without renal and cardiac disease, 83 males (59%) and 57 females (41%) with a mean age of 505 ± 160.5 months.

Serum creatinine was determined using Jaffe's alkaline picrate method. Blood urea nitrogen was determined using a urease UV test. Serum albumin was determined by the colorimetric brom cresol green (BCG) method. Twenty-four-hour urine was collected to determine proteinuria by pyrogallol-red colorimetric and Esbach methods. It was calculated by ISKDC references.[9]

Blood samples were collected from patients and controls and entered into EDTA. Genomic DNA from leukocytes was purified according to Miller.[10] The ACE I/D gene polymorphism was detected by PCR with primer sequences derived from Zee et al.[11] The sequences of the primers were chosen such that they flank the targeted region of the genome on the intron 16 of the ACE gene (17q23). The template DNA (0.4 mcg) was amplified using the following primers: forward, 5'CTGGAGAC-CACTCCCATCTTTCT-; and 3'reverse, 5'GATGTGGC-CATCACATT-CGTCAGA T 3'. To avoid mistyping between ID and DD, an I-specific primer pair, 5'- TGG-GACCACAGCGCCCGCCACTAC-3', and 5'- TCGC-CAGCCCTCCCATGCCCATAA-3', was also used to analyze all samples showing DD genotype. These primers (10 pmol of each) were added to a mixture containing 5 µl of 10X Cetus buffer (pH 8.3), 0.5 mM dNTP (dATP, dCTP, dGTP, dTTP), and 1.0 units of Taq DNA polymerase (Perkin Elmer Cetus).

PCR P Program (Perkin Elmer 9600 Thermal Cycler) was initiated in a final total volume of 50 ml. Thirty PCR amplification cycles consisted of a volume with thirty cycles, each made up of denaturation for 1 minute at 940°C, annealing for 1 minute at 58°C, and primer extension for 1 minute at 72°C. PCR products of ACE gene locus were examined by agarose gel electrophoresis (3% agarose) at 150 V for 60 minutes and visualized at room temperature under UV after ethidium bromide staining.

Statistical Method

In this study, all statistical analyses were performed using the SPSS 11.0 statistical program. Genotypes, allele frequency, clinical features at diagnosis were evaluated by chi-square test. Odds ratio and confidence intervals were calculated. Age and biochemical parameters were compared by Mann Whitney U test. Clinical data are reported as mean ± SD and percentage. Statistical significance was accepted as $p<0.05$.

RESULTS

All patients and controls were Turkish and ethnically homogeneous. The female and male ratios were 34% and 66% in patients and 41% and 59% in the control group, respectively. There was no statistically significant difference between two groups ($p>0.05$, $x^2=1.71$). The mean creatinine of the patients was 0.92 ± 0.77 mg/dL, the mean C3 values were 155.40 ± 62.38 mg/dL, the mean proteinuria values were 102.05 ± 64.75 mg/m^2/hour, and the serum albumin values were 2.5 ± 0.87 mg/dL.

The ACE genotype distribution was I/I, 4%; I/D, 78%; and D/D, 18% in the patient group, and I/I, 11%; I/D, 53%; and D/D, 36% in the control group. The distribution of the ACE I/D genotype in the patient group was statistically different from that of the control group ($p=0.004$, $x^2=11.2$). Sixty-four individuals have D/D genotype but 13 (20%) of them have the disease, 129 individuals have I/D genotype but 55 (42%) of them have the disease, and 18 individuals have I/I genotype but three (17%) of them have the disease. Carriers of I/D genotype introduce a 2.8-fold risk for nephrotic syndrome ($p=0.001$; OR, 2.8 [1.5–5.45]).

The study groups consisted of 52 (73%) with steroid-sensitive nephrotic syndrome (SSNS) and 19 (27%) with steroid-resistant nephrotic syndrome (SRNS). The distribution of ACE genotype was II, 6%; ID,75%; and DD, 19% in SSNS and ID, 84% and DD, 16% in SRNS. None of the patients had I/I genotype in the SRNS group. No statistically significant difference was found between steroid sensitivity and ACE genotypes ($p=0.5$, $x^2=1.33$).

DISCUSSION

Since 1990, the pathogenesis of cardiovascular and renal diseases were studied in genetic baseline, and the RAS, especially Ang II, was found to have an important role in renal scar development. It is supposed to act as an intrarenal growth hormone, inducing the proliferation of mesangial cells and an increase in glomerular volume, which has been shown to lead to subsequent glomerulosclerosis.

There is increasing evidence that the RAS is involved in the pathogenesis of progressive renal disease. Several recent studies have shown a significant association of the ACE gene and the progression of renal disease.[4,5]

ACE gene insertion/deletion polymorphism has been tested in many other diseases, such as diabetes mellitus, vesicoureteral reflux, and IgA nephropathy, and in most of these studies, associations with the incidence and/or severity of the disease were detected.[5–8]

In this study, no statistically significant difference was found between steroid sensitivity with ACE genotypes and alleles ($p>0.05$). However, none of the patients had I/I genotype in SRNS group. The polymorphism ratio on D/D was about 16% in patients with no responsive to steroid treatment. Patil et al. reported that steroid-sensitive patients showing a II genotype was more frequent than normal control. There was no significant difference in genotypes frequencies among steroid-sensitive groups.[12] In this study, there was a statistically significant difference in genotype polymorphism between patients and control ($p<0.05$). In this study, those having the D/D genotype showed a lower risk for being ill (OR=0.42, $p=0.01$). Lee et al. reported that patients who had the D/D genotype responded less to steroid therapy.[7] In this study, D/D genotype was 16% in the nonresponsive group of patients. Furthermore, the risk of getting the disease was lower in patients with I/I genotype, though this was not statistically significant (OR=0.3, $p=0.1$). This finding was similar to the one reported by Syrjanen et al., that I/I polymorphism was a good prognostic factor on Ig A nephritis.[13] On the other hand, Serdaroglu et al. reported that the DD genotype was more frequent in the SSNS group than that in control, and the distribution of the ACE genotype was similar among patients with FSGS and SSNS.[14] In this study, the carriers of the I/D genotype were found to introduce a 2.8-fold risk for nephrotic syndrome ($p=0.001$; OR, 2.8 [1.5–5.45]), but no similar findings about ACE genotypes were found in the literature. The authors believe that further studies are needed with a larger number of patients to confirm this result. Also, this finding may suggest that the association of ACE I/D genotypes with the development nephrotic syndrome may vary in different ethnic backgrounds.

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