Angiotensin-Converting Enzyme and Angiotensin Type 2 Receptor Gene Genotype Distributions in Italian Children with Congenital Uropathies

LUCIANA RIGOLI, ROBERTO CHIMENZ, CHIARA DI BELLA, EMANUELA CAVALLARO,
ROSARIO CARUSO, SILVANA BRIUGLIA, CARMELO FEDE, AND
CARMELO DAMIANO SALPIETRO

Department of Pediatrics [L.R., C.D.B., S.B., C.D.S.], Genetics Unit, University School of Medicine, 98125 Messina, Italy; Department of Pediatrics [R.C., C.F.], Nephrology Unit, University School of Medicine, 98125 Messina, Italy; Nephrology Unit [E.C.], Regina Apostolorum Hospital, 00041 Roma, Italy; and Department of Human Pathology [R.C.], University School of Medicine, 98125 Messina, Italy

ABSTRACT

Angiotensin I-converting enzyme (ACE) and angiotensin type 2 receptor (AT2R) gene polymorphisms have been associated with an increased incidence of congenital anomalies of the kidney and urinary tract (CAKUT). We investigated the genotype distribution of these polymorphisms in Italian children with CAKUT. We also evaluated the association between the ACE insertion/deletion and the AT2R gene polymorphisms with the progression of renal damage in subgroups of CAKUT patients. We recruited 102 Italian children with CAKUT; 27 with vesicoureteral reflux; 12 with hypoplastic kidneys; 20 with multicystic dysplastic kidneys; 13 with ureteropelvic junctions stenosis/atresia; 18 with nonobstructed, nonrefluxing primary megaureters; and 12 with posterior urethral valves and compared them with 92 healthy control subjects. ACE and AT2R gene polymorphisms were analyzed by PCR. The identification of AT2R gene polymorphisms in intron 1 and in exon 3 was revealed by enzymatic digestion. ACE genotype distribution in children with CAKUT was no different from that of the control subjects, but the subgroup of patients with radiographic renal parenchymal abnormalities showed an increased occurrence of the D/D genotype. The frequency of the G allele of AT2R gene in children with CAKUT was increased in respect to that of the control subjects. By contrast, no significant difference in the

frequency of the C and A alleles of the AT2R gene was found. Our findings indicate that the ACE gene can be a risk factor in the progression of renal parenchymal damage in CAKUT patients. Moreover, a major role of the AT2R gene in the development of CAKUT has been found, at least in Italian children. (*Pediatr Res* 56: 988–993, 2004)

Abbreviations

ACE, angiotensin I-converting enzyme
AT2R, angiotensin type 2 receptor
CAKUT, congenital anomalies of the kidney and urinary tract
HK, hypoplastic kidneys
I/D, insertion/deletion
MCDK, multicystic dysplastic kidneys
MU, nonobstructed, nonrefluxing primary megaureters
PUV, posterior urethral valves
Tc 99m DMSA, technetium 99m dimercaptosuccinate renal scintigraphy
Tc 99m DTPA, technetium 99m diethylenetriamine pentaacetic acid
UPJ, ureteropelvic junctions stenosis/atresia
VUR, vesicoureteral reflux

Congenital anomalies of the kidney and urinary tract (CAKUT) are now considered a major cause of renal failure in children (1). Ureteropelvic junction (UPJ) stenosis/atresia is the most common cause of a palpable abdominal mass in the newborn. Other forms of CAKUT include vesicoureteral reflux

(VUR); hypoplastic kidney (HK); multicystic dysplastic kidney (MCDK); nonobstructed, nonrefluxing primary megaureter (MU); and bladder outlet obstruction [e.g. posterior urethral valves (PUV)] (2). A number of well-recognized but puzzling features are associated with CAKUT. Anomalies such as UPJ stenosis/atresia and MCDK are often unilateral or highly asymmetrical.

Some of these anomalies are often concurrent (3–5). It is also noteworthy to mention that these abnormalities often take a familial pattern, showing incomplete and variable genetic penetrance. For this reason, it is believed that these assorted anatomic anomalies may share a common genetic cause (6–8).

Received March 4, 2003; accepted March 8, 2004.

Correspondence: Luciana Rigoli, M.D., Department of Pediatrics, University School of Medicine, Messina, Italy; e-mail: luciana.rigoli@unime.it

The authors greatly thank the Ministero dell'Università e della Ricerca Scientifica (Italy) for financial support.

DOI: 10.1203/01.PDR.0000145252.89427.9E

Recent genetic analyses on several families have identified several different chromosomal loci that are associated with CAKUT (9,10), although no specific gene involved has been identified in these studies.

Angiotensin I-converting enzyme (ACE) is one of the most intensely studied candidate genes in human renal malformations (11–13). The insertion or deletion (I/D) of a 287-bp fragment in intron 16 of the ACE gene has proved to influence the activity of the renin-angiotensin system (14) and, therefore, the production of angiotensin II. ACE I/D gene polymorphism is a risk factor for renal parenchymal damage in patients with congenital urologic abnormalities and seems particularly relevant in children with VUR, where it is an independent predisposing factor (15–19). However, it was recently observed that mice, especially males, with a disrupted angiotensin type 2 receptor (AT2R) gene, which is located on the X chromosome, often have a range of congenital anomalies of the kidney and urinary tract and that white male patients with UPJ and MCDK frequently have A→G transition in intron 1 of the AT2R gene (20,21).

The AT2R is considered to be associated with the development of fetal organs (22–24). Recently, Ichikawa and colleagues (2) proposed the molecular basis for the classical ureteral bud theory. According to this theory, abnormalities in AT2R expression hinder the interaction between the ureteric bud and metanephric blastema and hamper the normal development of the nephron and ureter, accompanied with the development of CAKUT (25).

The aim of the present report was to examine the genotype distribution of the ACE I/D gene polymorphism and of two polymorphic sites of the AT2R gene in Italian children with CAKUT. Moreover, we evaluated the possible association between the ACE I/D gene and the AT2R gene polymorphisms with the progression of renal damage in subgroups of CAKUT patients.

METHODS

A total of 102 Italian children (45 girls and 57 boys; age range 6 mo–15 y; median age 9.5 y) with defined VUR, HK, MCDK; UPJ stenosis/atresia, MU; and bladder outlet obstruction (*e.g.* PUV) were studied. The patients, coming from Sicily and from some regions of Calabria (Italy), were recruited from the Paediatrics Department at the University of Messina (Italy) between April 2000 and April 2002. The study was approved by the ethics committee of the University of Messina. Parents gave their written informed consent. Personal and family history revealed that only six of the 102 children had a positive family history for CAKUT. The clinical data included the patients' history; physical examination; and radiologic investigations, including renal ultrasonography, technetium 99m dimercaptosuccinate (Tc 99m DMSA) renal scintigraphy, technetium 99m diethylenetriamine pentaacetic acid (Tc 99m DTPA), cystosonography, cystourethrography, and *i.v.* pyelogram (IVP). Blood for genotyping was obtained by venipuncture.

VUR was diagnosed by voiding cystosonography and Tc 99m DMSA for the evaluation of the parenchymal scar. The cystourethrography was used to analyze the entity of the reflux.

Diagnosis of reflux was classified from grade 1 to 5 according to the International Reflux Classification (26). HK was defined by ultrasonography, cystosonography, and Tc 99m DMSA. By ultrasonography, the kidneys appeared smaller than they should be for the patient's age. DMSA renoscintigraphy revealed a small functioning kidney. Therefore, a diagnosis of HK was made after a small-sized kidney persisted for >6 mo on DMSA renal scintigraphy and ultrasound. The ultrasound scan and the Tc 99m DTPA with furosemide test were used to carry out the diagnosis of UPJ. Primary MU was defined by renal ultrasonography, Tc 99m DTPA, and cystourethrography. PUV was diagnosed by cystourethrography.

In each patient, renal parenchymal abnormalities observed through ultrasonography were confirmed by DMSA renoscintigraphy. We use the terms *radiographic lesions* and *renal parenchymal lesions* to denote these radiographically observed renal abnormalities.

The control group consisted of 92 Italian children who were recruited from inpatient and outpatient services of the University of Messina (Italy) and showed no evidence of clinical renal disease and had a normal renal function and no renal abnormalities documented by ultrasonography. Patients or control subjects with any chronic diseases or syndromic disorders were excluded. Blood for genotyping was obtained by venipuncture.

Patient Group

Primary VUR. This group included 27 patients, 12 boys and 15 girls (median age 9.0 y at last evaluation). The left side was affected in eight patients, the right side was affected in nine patients, and bilateral reflux was present in 10 patients. According to International Reflux Classification (26), the patients were classified from grade 1 to 5 as follows: two patients (one boy and one girl) with grade 1; 11 patients (six boys and five girls) with grade 2; seven patients (three boys and four girls) with grade 3, and seven patients (four boys and three girls) with grade 4 (Table 1).

HK

Twelve patients (eight boys and four girls) were affected by HK. In particular, the HKs were unilateral in seven and bilateral in five patients. None of the seven children had anomalies in the contralateral kidney.

MCDK

Twenty patients (11 boys and nine girls) were affected by MCDK.

Table 1. Clinical characteristics of the patient group

CAKUT	No. of patients		
	Total	Male	Female
Primary VUR	27	12	15
HK	12	8	4
MCDK	20	11	9
UPJ	13	8	5
Primary MU	18	15	3
PUV	12	12	—

UPJ

This group included 13 patients, eight boys and five girls (median age 7.5 y at last evaluation). The left side was affected in five patients, the right side was affected in six patients, and bilateral UPJ was present in two patients.

PRIMARY MU

This group included 18 patients, 15 boys and three girls, with confirmed diagnosis of primary MU (median age 8.0 y at last evaluation). The left side was affected in 10 patients, the right side was affected in five patients, and bilateral MUs were observed in three patients. None of the patients with bilateral MU was affected by Eagle-Barrett syndrome.

PUV

Twelve male patients were affected by PUVs (median age 6.0 y at last evaluation). This group also included patients with PUVs and secondary reflux ($n = 4$) or secondary obstructive megaureters ($n = 5$) or obstructive/refluxive megaureters ($n = 3$).

CONTROL SUBJECTS

The control group consisted of 92 Italian children of the University of Messina (Italy) without any chronic diseases or urological malformations, confirmed by ultrasonography in every patient (median age 12.0 y).

Determination of ACE Genotypes

DNA was isolated from 200 μ L of whole-blood samples. PCR amplification to detect ACE I/D polymorphism was carried out using the following primers: 5'-CTGGAGACCACTCCCATCCTTTCT-3' and 5'-GATGTGGCCATCACATTCGTCAGAT-3'. Amplification with this primer pair produces products of ~490 and 190 bp, corresponding to the I and D alleles, respectively. Thermocycling consisted of denaturation at 94°C for 60 s, annealing at 65°C for 60 s, and extension at 72°C for 90 s for 32 cycles, followed by a final extension at 72°C for 7 min. PCR products (10 μ L) were mixed in 2 μ L of glycerol-based loading buffer, size fractionated by electrophoresis 0.8% agarose gel that contained 1 mg/mL of ethidium bromide, and visualized by UV transillumination. Because the D allele is preferentially amplified, mistyping of I/D heterozygous as D homozygotes is possible (27). For eliminating this possibility, all D/D samples were re-amplified with an insertion-specific primer pair, which recognizes the inserted sequence, 5'-TGGGACCACAGCGCCCGCCACTAC-3' and 5'-TCGCCAGCCCTCCCATGCCCATAA-3' (28). Thermocycling consisted of 35 cycles of denaturation at 94°C for 1 min, and annealing/extension at 78°C for 1 min, followed by a final extension at 72°C for 10 min. We found that the false-positive rate was 13%, which is similar to the 10% previously found by other investigators (29).

Determination of AT2R Genotypes

The subjects were analyzed for the two polymorphisms so far reported in the AT2R gene (21,30). For analyzing the

polymorphism in intron 1, genomic DNA was amplified by PCR in a 20- μ L reaction mixture that contained 20 mM of Tris-HCl (pH 8.0), 50 mM of KCl, 2 mM of MgCl₂, 0.2 mM of each of four deoxynucleotide triphosphates, 1 unit of DNA polymerase (EuroTaq; Euroclone, Paignton, UK), and 100 ng of genomic DNA using 0.4 mM of each of a primer pair of 5'-GGAAAGTAGAACATACATTAAATG-3' and 5'-CCTGTAAGAGAAACAGCAGCTAAAGAATT-3'. Thermocycling in a GeneAmp PCR System 9600 (Perkin-Elmer) consisted of 5' of preincubation at 95°C, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 1 min, extension at 72°C for 1 min, and final extension of 7 min at 72°C. PCR products were digested with the restriction enzyme *Eco*RI (New England Biolabs, Beverly, MA) for 1 h at 37°C. The A→G transition yielded the *Eco*RI digestion site, thus providing restriction fragment length polymorphism.

The polymorphism in a 3' untranslated region of exon 3 was examined by using the following primers: 5'-GGATTCA-GATTTCTCTTTGAA-3' and 5'-GCATAGGAGTATGATT-TAATC-3'. Thermocycling consisted of 5 min of preincubation at 95°C, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, and final extension of 7 min at 72°C. PCR products were digested with the restriction enzyme *Alu*I (New England Biolabs). C→A transition lost the *Alu*I digestion site, thus yielding restriction fragment length polymorphism.

Statistical Analysis

Univariate and multivariate data analyses were performed using STATA 7 (31). The statistical methods used were the χ^2 test and the Fisher exact probability test. A two-tailed $p < 0.05$ was taken as the level of significance.

RESULTS

All clinical, radiographic, and follow-up data were available in 102 patients. Twenty-seven patients had primary VUR (12 boys and 15 girls); 12 (eight boys and four girls) had HKs; 20 (11 boys and nine girls) had MCDKs; 13 (eight boys and five girls) had UPJ stenosis/atresia; 18 (15 boys and three girls) had primary MUs, and 12 boys had PUVs (Table 1). The male-to-female ratio was 0.8 in primary VUR, 2.0 in HK, 1.2 in MCDK, 1.6 in UPJ, and 5.0 in primary MU. In most patients, the diagnosis was carried out in the first year of life, but in 38 of the 102 patients, the diagnosis was performed later than the first year of life.

In the VUR patients, ultrasonic and nuclear medicine studies showed several areas of dysmorphism loosely called "scars" that occur even in the absence of infection. Moreover, 60% of the children with grade 2 and 47% with grade 3 had abnormal kidneys, indicating that even in low grades of reflux, parenchymal defects were common.

In children with VUR, three major types of abnormal states were found by DMSA: dysplasia (37%), medial defects (38%), and polar defects (25%). In particular, we identified three patterns of renal damage: overall decreased uptake of renal radionuclide that was 20 to 35% of relative uptake, focal

defects in uptake, and shrunken kidney with relative uptake <20%.

In the HK patients, ultrasound studies demonstrated a smaller, underdeveloped, and smooth-surfaced kidney than the contralateral one. Its parenchyma was uniformly thin. There was a compensatory enlargement of the other kidney. On DMSA renoscintigraphy, the hypoplastic kidney showed a small function.

MCDK was diagnosed as multiple cysts on ultrasound scan and without any function on Tc 99m DMSA. They were of varying size with little intervening stroma and lacking a normal reniform shape and a normal collecting system. The largest cysts were located in the periphery. The cysts were noncommunicating, round, anechoic; they had smooth, thin walls and showed distal acoustic enhancement. In 11 patients they were so extensive that they caused deformation of the kidney. Distortion of the collecting system occurred in seven patients.

Thirty-three percent of the patients had contralateral renal malformations. VUR was common (nine patients). Reflux occurred into the contralateral ureter in eight patients and into the ipsilateral atretic ureter in five of them (two had bilateral reflux). Four patients had ectopic ureters: three cases were ipsilateral and inserted into the prostatic urethra (two cases, both with reflux) or into the seminal vesicle (one case); one case was contralateral and inserted into the bladder neck. Four patients had an ipsilateral obstructed single-system ureterocele. Four patients had bladder diverticula. Other abnormalities included hypospadias, opacification of a small utricle (not in the patient with hypospadias), ejaculatory duct reflux, internal urachal sinus, and lipomeningocele with a neurogenic bladder. DMSA renal scanning showed an absence of function in the kidney with MCDK.

In one case of severe hydronephrosis, a peripheral rim of activity was observed, followed by an increase in excretion noted in delayed images. Delayed images also showed retention of the DTPA in the pelvocaliceal system with little or no washout with furosemide.

In the UPJ, the primary investigation was ultrasound. This pointed out an enlarged renal pelvis with or without enlargement of the calyces (eight and five patients, respectively). Ultrasound features of unilateral UPJ obstruction included varying degrees of pelvocaliectasis without any evidence of ureterectasis; ectopic ureterocele; dilated, thick-walled urinary bladder; or posterior ureteral dilation.

In four patients the ureter was of normal caliber, easily seen behind the bladder. DTPA pointed out an inadequate drainage of the kidney on the time activity curves with images showing retention within the kidney with no ureteric dilation.

MU was found in 18 patients and was associated with pelvicaliceal dilation. In 10 patients, the DTPA scanning revealed a good differential renal function. Two of these children later developed a slight deterioration in function. Twelve patients, after a period ranging from 4 mo to 5 y, showed partial or complete regression of ureteral dilation with good stable renal function.

The diagnosis of PUV was suggested by the finding of hydronephrosis (expansion and enlargement of the kidney). In the PUV patients, the cystourethrogram characteristically re-

vealed the valve leaflets within the urethra, an enlarged posterior portion of the urethra, and a thickened bladder wall, the last result pointing out elevated bladder pressure that is secondary to the obstruction of the urine flow.

ACE I/D genotype distribution. The ACE I/D genotype distribution in the control group was as follows: I/I 26.0%, I/D 52.0%, and D/D 22.0%, which is in agreement with the genotype frequency in other groups of white populations ($p = \text{NS}$) (22). Among all of the patients with congenital uropathies, the ACE genotype distribution was as follows: I/I 15.0%, I/D 54.0%, and D/D 31.0%, which was no different from the control population (Fig. 1).

Of the 102 patients, 73 had radiographic demonstrable renal parenchymal lesions. Renal lesions were seen in 25 patients with primary VUR, in eight with HK, in 20 with MCDK, in eight with UPJ, in nine with MU, and in 10 with PUV. The ACE genotype distribution in these patients with radiographically abnormalities was as follows: I/I 10.0%, I/D 52.0%, and D/D 38.0%, which was significantly different from the normal control subjects, showing increased occurrence of the D/D genotype ($p < 0.001$, $\chi^2 = 15.1$; Fig. 2). Moreover, the genotype distribution was different between patients with urological abnormalities and radiographic lesions (group A) and patients with urological abnormalities but no parenchymal lesions (group B; $p < 0.05$, $\chi^2 = 6.4$; Fig. 3).

Because the ACE genotype has been identified as a risk factor for progressive damage from a primary glomerular disease, we evaluated at a later stage whether it is also a risk factor for the development of parenchymal damage associated with CAKUT. Therefore, we analyzed the genotype of patients who initially had normal radiographs and later developed parenchymal lesions, *i.e.* we excluded patients with lesions at presentation, because these may have reflected fetal maldevelopment of the kidneys. There were 13 patients in this subgroup. There was a remarkable overrepresentation of the D/D genotype. In this group, the I/I, I/D, and D/D genotype was 0, 41, and 59%, respectively. It is important to note that the duration of follow-up of these patients did not differ from those who did not develop renal lesions (mean 7.5 *versus* 6.9 y; $p = \text{NS}$).

AT2R genotype distribution. Analysis of PCR–restriction fragment length polymorphism showed that the frequency of A and G alleles at the A→G transition site (position –1332 from the translation initiation site) of intron 1 was 64 (69.6%) and 28 (30.4%), respectively, among the general Italian population. The children with CAKUT showed a significant statistical difference ($p < 0.05$, $\chi^2 = 4.7$) in the frequency of the G allele

Figure 1. ACE I/D genotype distribution in children with CAKUT and control subjects. Patients: I/I 15%; I/D 54%; D/D 31%. Control subjects: I/I 26%; I/D 52%; D/D 22%. The ACE genotype distribution of the CAKUT patients was no different from the control population.

Figure 2. ACE I/D genotype distribution in CAKUT children with parenchymal lesions and control subjects. Patients: I/I 10%; I/D 52%; D/D 38%. Control subjects: I/I 26%; I/D 52%; D/D 22%. The ACE genotype distribution of the CAKUT children with parenchymal lesions was significantly different from control subjects, showing increased occurrence of the D/D genotype ($p < 0.001$, $\chi^2 = 15.1$).

Figure 3. ACE I/D genotype distribution in CAKUT children with and without parenchymal lesions. Patients with renal parenchymal lesions: I/I 10%; I/D 52%; D/D 38%. Patients without renal parenchymal lesions: I/I 22%; I/D 52%; D/D 26%. The genotype distribution was different between CAKUT children with urologic abnormalities and radiographic lesions and patients with urologic abnormalities but no parenchymal lesions ($p < 0.05$, $\chi^2 = 6.4$).

when compared with the general population: 48 (47.0%) in a total of 102 patients. These patients were categorized specifically as 14 (51.8%) of 27 in VUR, seven (58.3%) of 12 in HK, 11 (55.0%) of 20 in MCDK, four (30.7%) of 13 in UPJ, six (33.3%) of 18 in MU, and six (50.0%) of 12 in PUV. No significant difference of the genotype distribution was observed in the various groups of congenital uropathies. There was no significant difference in the frequency of the C and A alleles at the C→A transition site (position 1593) of exon 3 between the control group and the children with CAKUT ($p = \text{NS}$).

DISCUSSION

Congenital urologic abnormalities are common causes of renal failure in children, contributing to ~25% of chronic renal failure in this age group. Although the outlook relates to the severity of initial renal damage, there is considerable heterogeneity in renal parenchymal destruction among individuals, and the reasons for this heterogeneity are not fully understood.

Recently, it was reported that ACE I/D gene polymorphism is a risk factor for renal parenchymal damage in patients with congenital urologic abnormalities (32). We have determined the incidence of the ACE gene I/D polymorphism in 102 Italian children with different urologic abnormalities (CAKUT) leading to variable degrees of renal impairment and in 92 healthy control subjects with a similar ethnic background. ACE genotype distribution did not differ between CAKUT patients and normal control subjects. Indeed, several authors found that an experimental increase in the ACE gene copy number and consequently the increase in ACE activity were not associated with an increased incidence of urologic abnormalities in transgenic mice (33). In our work, this factor was also true when we

examined the total population of CAKUT patients and compared them with normal control subjects. By contrast, when we analyzed the CAKUT patients, who showed radiographic renal parenchymal damage, the D/D genotype seemed to be slightly overrepresented with respect to subjects who were affected by congenital uropathies but without any renal damage. This was also evident in patients who started out with normal kidneys but developed renal parenchymal damage over time. Notably, there was no difference in the duration of follow-up between patients who developed scars and those who did not.

It has been reported that AT2R gene null mutant mice display CAKUT (20,22). In a study on the human AT2R gene, a functionally significant mutation was seen within the so-called “branch point” of intron 1, which resulted in a significant alteration in the quality and the quantity of the AT2R mRNA as a result of abnormal mRNA splicing. This mutation could be responsible of an alteration in the AT2R gene expression and function during renal development (21).

Screening of this human gene revealed in about one third of the white American population an A→G transition at position -1332, the site of the “branch point” motif within intron 1. By means of direct DNA sequencing, it was found that the mRNA with the A genotype consists of exons 1, 2, and 3, whereas mRNA with the G genotype directly follows exon 1. Moreover, the amount of abnormally spliced mRNA with the G genotype was markedly lower than that of normally spliced mRNA with the A genotype (21,34). Recently, a C→A transition at position np 1593 in exon 3 of AT2R gene was identified, but there are contrasting data regarding its function (30,35).

A few independent DNA studies on American and German populations of CAKUT patients revealed a significant correlation between the incidence of CAKUT and the specific A→G mutation in intron 1 (21). On the contrary, no evidence for this AT2R gene derangement has been found in the Japanese population (35).

We analyzed the frequency of the AT2R gene polymorphisms at the A→G transition site of intron 1 and at the C→A transition site (position 1593) of exon 3 in Italian children with congenital uropathies. We also evaluated the ACE I/D polymorphism and the distribution of the different genotypes of AT2R gene in the subgroups of CAKUT to establish whether there is an association between these polymorphisms and the progression of renal damage.

To our knowledge, this is the first Italian study in which the incidence of the AT2R gene A→G and C→A polymorphisms has been investigated in children with CAKUT. We found an increased frequency ($p < 0.05$) of the AT2R gene A→G polymorphism in Italian CAKUT patients when compared with the control subjects. However, the frequency of this polymorphism in CAKUT patients was lower than those reported by Nishimura *et al.* (21). The discrepancy between these studies could be explained by the differences in genetic and ethnic backgrounds.

The present study found no significant difference in the incidence of the A→G transition between the CAKUT patients with and without renal parenchymal damage. Moreover, no significant association of the AT2R gene C→A transition site with CAKUT was observed in our patients.

The number of cases in the present study seems to be too low to reach any definitive conclusions. However, CAKUT patients are very rare and our results are encouraging enough to amplify our study with a greater number of cases.

REFERENCES

1. Woolf AS, Winyard PJ 1998 Advances in the cell biology and genetics of human kidney malformations. *J Am Soc Nephrol* 38:1114–1125
2. Pope JC, Brock JW, Adams MC, Stephens FD, Ichikawa I 1999 How they begin and how they end. Classic and new theories for the development and deterioration of congenital anomalies of the kidney and urinary tract, CAKUT. *J Am Soc Nephrol* 10:2018–2028
3. Atiyeh B, Husmann D, Baum M 1992 Contralateral renal abnormalities in multicystic-dysplastic kidney disease. *J Pediatr* 121:65–67
4. Squiers EC, Morden RS, Bernstein J 1987 Renal multicystic dysplasia: an occasional manifestation of the hereditary renal adysplasia syndrome. *Am J Med Genet Suppl* 3:279–284
5. Peters CA, Carr MC, Lais A, Retik AB, Mandell J 1992 The response of the fetal kidney to obstruction. *J Urol* 148:503–509
6. Izquierdo L, Porteous M, Paramo PG, Connor JM 1992 Evidence for genetic heterogeneity in hereditary hydronephrosis caused by pelvi-ureteric junction obstruction, with one less locus assigned to chromosome 6p. *Hum Genet* 89:557–560
7. Fryns JP, Kleczkowska A, Moerman P, Vandenbergh K 1993 Hereditary hydronephrosis and the short arm of chromosome 6. [letter] *Hum Genet* 91:514–515
8. Groenen PM, Garcia E, Debeer P, Devriendt K, Fryns JP, Van de Ven WJ 1996 Structure, sequence, and chromosome 19 localization of human *USF2* and its rearrangement in a patient with multicystic renal dysplasia. *Genomics* 38:141–148
9. Sanyanusin P, Schimmenti LA, McNoe LA, Ward TA, Pierpont ME, Sullivan MJ, Dobyns WB, Eccles MR 1995 Mutation of the *PAX2* gene in a family with optic nerve colobomas, renal anomalies, and vesicoureteral reflux. *Nat Genet* 9:358–364
10. Schiavello T, Burke V, Bogdanova N, Jasik P, Melsom S, Boudville N, Robertson K, Angelicheva D, Dworniczak B, Lemmens M, Horst J, Todorov V, Dimitrakov D, Sulowicz W, Krasniak A, Stompor T, Beilin L, Hallmayer J, Kalaydjieva L, Thomas M 2001 Angiotensin-converting enzyme activity and the ACE Alu polymorphism in autosomal dominant polycystic kidney disease. *Nephrol Dial Transplant* 16:2323–2327
11. Hohenfellner K, Wingen AM, Nauroth O, Wuhl E, Mehls O, Schaefer F 2001 Impact of ACE I/D gene polymorphism on congenital renal malformations. *Pediatr Nephrol* 16:356–361
12. Yoneda A, Oue T, Puri P 2001 Angiotensin-converting enzyme genotype distribution in familial vesicoureteral reflux. *Pediatr Surg Int* 17:308–311
13. al-Eisa A, Haider MZ, Srivastva BS 2000 Angiotensin-converting enzyme gene insertion/deletion polymorphism and renal damage in childhood uropathies. *Pediatr Int* 42:348–353
14. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F 1990 An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 86:1343–1346
15. Schunkert H 1997 Polymorphism of the angiotensin-converting enzyme gene and cardiovascular disease. *J Mol Med* 75:867–875
16. Ozen S, Alikasifoglu M, Tuncbilek E, Bakkaloglu A, Besbas N, Aran B, Saatci U 1997 Polymorphisms in angiotensin converting enzyme gene and reflux nephropathy: a genetic predisposition to scar formation? *Nephrol Dial Transplant* 12:2031–2032
17. Harden PN, Geddes C, Rowe PA, McLroy JH, Boulton-Jones M, Rodger RS, Junor BJ, Briggs JD, Connell JM, Jardine AG 1995 Polymorphisms in angiotensin-converting enzyme gene and progression of IgA nephropathy. *Lancet* 345:1540–1542
18. Marre M, Jeunemaitre X, Gallois Y, Rodier M, Chatellier G, Sert C, Dusselier L, Kahal Z, Chaillous L, Halimi S, Muller A, Sackmann H, Bauduceau B, Bled F, Passa P, Alhenc-Gelas F 1997 Contribution of genetic polymorphism in the renin-angiotensin system to the development of renal complications in insulin-dependent diabetes. *J Clin Invest* 99:1585–1595
19. Ohtomo Y, Nagaoka R, Kaneko K, Fukuda Y, Miyano T, Yamashiro Y 2001 Angiotensin converting enzyme gene polymorphism in primary vesicoureteral reflux. *Pediatr Nephrol* 16:648–652, 2001A
20. Koike G, Horiuchi M, Yamada T, Szpirer C, Jacob HJ, Dzau VJ 1994 Human type 2 angiotensin II receptor gene: cloned, mapped to the X chromosome, and its mRNA is expressed in the human lung. *Biochem Biophys Res Commun* 203:1842–1850
21. Nishimura H, Yerkes E, Hohenfellner K, Miyazaki Y, Ma J, Hunley TE, Yoshida H, Ichiki T, Threadgill D, Phillips JA 3rd, Hogan BM, Fogo A, Brock JW 3rd, Inagami T, Ichikawa I 1999 Role of the angiotensin type 2 receptor gene in congenital anomalies of the kidney and urinary tract, CAKUT, of mice and men. *Mol Cell* 3:1–10
22. Nouet S, Nahmias C 2000 Signal transduction from the angiotensin II AT2 receptor. *Trends Endocrinol Metab* 11:1–6
23. Zemel S, Millan MA, Feuillan P, Aguilera G 1990 Characterization and distribution of angiotensin II receptors in the primate fetus. *J Clin Endocrinol Metab* 71:1003–1007
24. Kakuchi J, Ichiki T, Kiyama S, Hogan BL, Fogo A, Inagami T, Ichikawa I 1995 Developmental expression of renal angiotensin II receptor genes in the mouse. *Kidney Int* 47:140–147
25. Pryde PG, Sedman AB, Nugent CE, Barr M Jr 1993 Angiotensin-converting enzyme inhibitor fetopathy. *J Am Soc Nephrol* 3:1575–1582
26. International Reflux Study Committee 1981 Medical versus surgical treatment of primary vesicoureteral reflux. Report of the International Reflux Study Committee. *Pediatrics* 67:392–400
27. Shanmugam V, Sell KW, Saha BK 1993 Mistyping ACE heterozygotes. *PCR Methods Appl* 3:120–121
28. Lindpaintner K, Pfeffer MA, Kreutz R, Stampfer MJ, Grodstein F, LaMotte F, Buring J, Hennekens CH 1995 A prospective evaluation of an angiotensin-converting-enzyme gene polymorphism and the risk of ischemic heart disease. *N Engl J Med* 332:706–711
29. Hunley TE, Julian BA, Phillips JA 3rd, Summar ML, Yoshida H, Horn RG, Brown NJ, Fogo A, Ichikawa I, Kon V 1996 Angiotensin converting enzyme gene polymorphism: potential silencer motif and impact on progression in IgA nephropathy. *Kidney Int* 49:571–577
30. Katsuya T, Horiuchi M, Minami S, Koike G, Santoro NF, Hsueh AJ, Dzau VJ 1997 Genomic organization and polymorphism of human angiotensin II type 2 receptor: no evidence for its gene mutation in two families of human premature ovarian failure syndrome. *Mol Cell Endocrinol* 127:221–228
31. Stata Corp 2001 Stata Statistical Software Release 7.0. Stata, College Station, TX
32. Hohenfellner K, Hunley TE, Brezinska R, Brodhag P, Shyr W, Brenner W, Habermehl P, Kon V 1999 Ace I/D gene polymorphism predicts renal damage in congenital uropathies. *Pediatr Nephrol* 13:514–518
33. Krege JH, Kim HS, Moyer JS, Jennette JC, Peng L, Hiller SK, Smithies O 1997 Angiotensin converting enzyme gene mutations, blood pressures, and cardiovascular homeostasis. *Hypertension* 29:150–157
34. Miyazaki Y, Ichikawa I 2001 Role of the angiotensin receptor in the development of the mammalian kidney and urinary tract. In: *Comparative Biochemistry and Physiology*. Elsevier Science Inc, Baltimore, Part A 128:89–97
35. Hiraoka M, Taniguchi T, Nakai H, Kino M, Okada Y, Tanizawa A, Tsukahara H, Ohshima Y, Muramatsu I, Mayumi M 2001 No evidence for AT2R gene derangement in human urinary tract anomalies. *Kidney Int* 59:1244–1249