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Annales de Génétique 45 (2002) 123–126

ANNALES DE GÉNÉTIQUE

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Original Article

Non-allelic heterogeneity in familial unilateral renal adysplasia

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Received 9 April 2002; accepted 25 July 2002

Abstract

We report three families with dominant unilateral renal adysplasia without vesico-ureteral reflux. No dysmorphia or anomalies were evident in the reproductive system. Ophthalmological examination excluded the presence of optic nerve coloboma or other ocular anomalies. No mutations were detected in the *EMX*$_2$ and in *PAX*$_2$ genes of affected members. Other homeobox genes could be responsible for this anomaly in these three families.

Keywords: Renal adysplasia; EMX$_2$ gene; PAX$_2$ gene; Homeobox genes; Renal malformation

1. Introduction

Unilateral renal adysplasia (URA) is a relatively common malformation of the urinary tract that is usually diagnosed during fetal ultrasonography (US) [1]. URA may be isolated or it may be associated with chromosomal (X-linked Kallmann'syndrome) [2] or non-chromosomal syndromes that include the *VATER/VACTERL* (vertebral anomalies, cardiac defects, tracheo-oesophageal fistula) [3], *MURCS* (mullerian duct aplasia, renal aplasia, cervico thoracic somite malformation) associations [4,5]. A sporadic patient with unilateral iris coloboma, microphthalmia, URA and renal hypoplasia was reported in 1992 [6].

Familial URA is a rare condition that is inherited as an autosomal dominant trait with incomplete penetrance and variable expressivity [1,7]. Evidence is now accumulating to suggest a role for certain genes (*EMX*$_2$ and *PAX*$_2$ genes) in the control of nephrogenesis [8–12].

In this report we describe three Sicilian families in which URA was present in two consecutive generations. Sequence analysis of DNA from all affected patients was undertaken to identify possible mutations in the *EMX*$_2$ and *PAX*$_2$ genes.

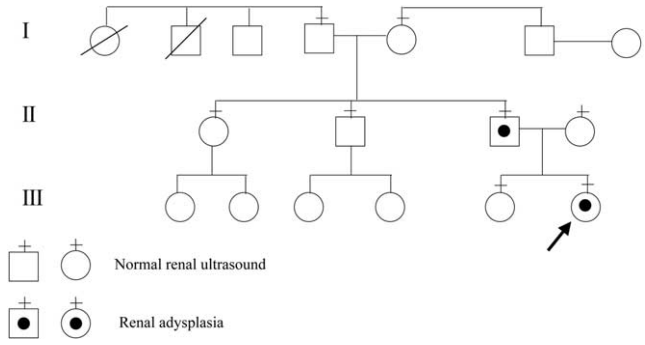

Fig. 1. Family 1: Normal renal ultrasound. Renal adysplasia.

2. Case reports

2.1. Family 1

A 7-month-old girl, III.6 (Fig. 1), was admitted to the Day Hospital of our Pediatric Department for low weight since birth. At admission, her weight was below the third percentile but all routine laboratory tests were normal. Renal US revealed URA on the left side. Her 42-year-old father, II.2, showed URA on the right side with no signs of renal disease.

A renal scintigraphy confirmed the diagnosis of URA in the proband and her father. A contrast voiding cystography demonstrated the absence of vesico-ureteral reflux in both

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PII: S0003-3995(02)01132-2

subjects. Ophthalmological examination excluded in both patients the presence of optic nerve coloboma or other ocular anomalies. No dysmorphia or anomalies were evident in the reproductive system as indicated by US of the proband and her father.

Other available relatives (I.4, I.5, II.1, II.2, II.4 and III.5) revealed normal kidneys by US examination.

2.2. Family 2

A 34-year-old woman, II.8 (Fig. 2), underwent US during pregnancy and was found to have URA on the right side. Her son, III.1, 15 days after birth, had a urinary tract infection and renal US revealed URA on the right side. At admission, all routine laboratory tests were normal. Renal scintigraphy confirmed the diagnosis of URA in the proband and in her son. In both no signs of impairment in the renal function were revealed. No vesico-ureteral reflux was demonstrated in both patients when contrast voiding cystography was carried out. No dysmorphia or anomalies in the reproductive system as indicated by US were noted in the proband and in her son. At the ophthalmological examination the presence of optic nerve coloboma and other ocular anomalies were excluded.

Examination by US of all other available relatives (I.1, II.7, II.9, II.10, II.11 and II.12) revealed normal kidneys.

2.3. Family 3

A 11-month-old girl, III.5 (Fig. 3), showed URA on the right side during a renal US performed after repeated urinary tract infections. Unilateral and smaller ectopic kidney on the right side was diagnosed in her father, II.5, by renal US. Renal scintigraphy confirmed both diagnoses. No renal function impairment was demonstrated. A contrast voiding cystography performed in both patients did not show any vesico-ureteral reflux. The reproductive system and the ophthalmological examination showed no anomalies in both subjects.

In all other available relatives (I.1, I.2, II.6, and III.4), US revealed normal kidneys.

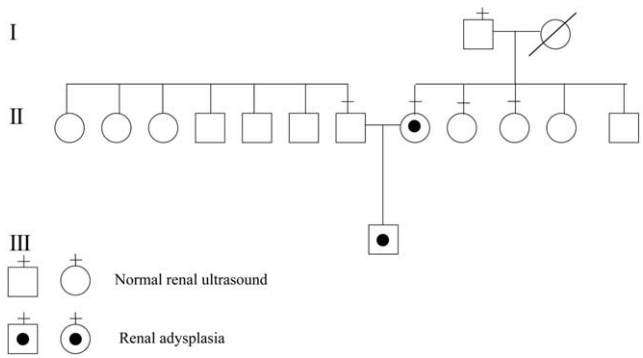

Fig. 2. Family 2: Normal renal ultrasound. Renal adysplasia.

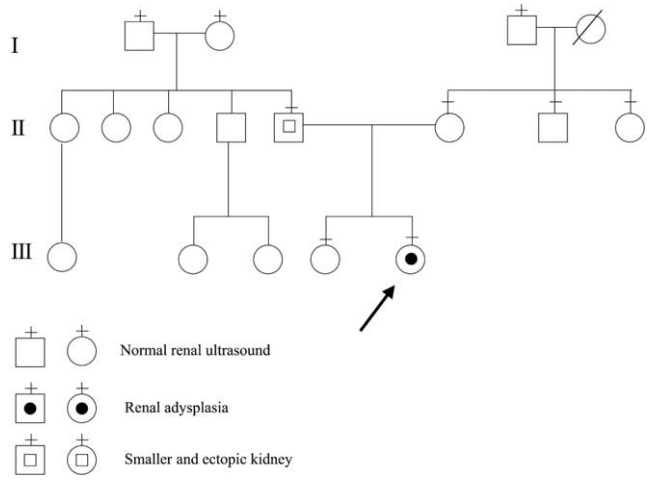

Fig. 3. Family 3: Normal renal ultrasound. Renal adysplasia. Smaller and ectopic kidney.

2.4. Genetic studies

2.4.1. EMX_2

The human EMX_2 is composed of three exons (Fig. 4) of 374, 185 and 165 bp, respectively. We performed the sequence analysis of the three exons on DNAs of all individuals reported in the pedigrees, according to a method described elsewhere [13]. The following couples of oligonucleotides were used to amplify the exons:

exon 1: E2.1/1: 5' ACAAACGAGTCCCCAATTCTCGTCC 3';
E2.1/4: 5' CTGCAGTTCCGCGACGTGGCACGT 3'.

exon 2: E2.2/1: GGTCAGAGCAGCCCCCCCTAATGG 3';
E2.2/2: 5' GGCGTGGAACCAGCTACCAGGACC 3'.

exon 3: E2.3/1: 5' GAAAGAACTAACGCACCCCATCTGCC 3';
E2.3/2: 5' CACCTCTCCCTGTCTCTTTTCCTCCA 3'.

The reaction mixture was: genomic DNA (200 ng), $MgCl_2$ (1.5 mM), dNTPs (1 μM), TaqGold (Perkin Elmer Cetus, 1 Unit/reaction), reaction buffer (1X), primers (1.5 mM). The amplification was performed as follows: 1 cycle at 95 °C/10 min; 35 cycles at: 95 °C/1 min, 75 °C/45 s, 72 °C/1 min. The fragments from PCR were purified using the Quiagen kit "QUIAIEX II" gel extraction kit (cat. no. 20021) and sequenced using the "Thermo Sequenase Radiolabeled Terminator Cycle Sequencing Kit" from USB. This method allow us to distinguish the heterozygous patients from homozygous ones. We did not consider the intronic regions in this analysis. None of the analyzed samples showed mutations in the coding region.

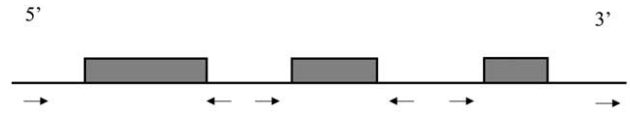

Fig. 4. Schematic representation of the EMX_2 gene. The three exons are showed as grey boxes. The intronic regions are not in scale. The arrowhead are the regions where the primers were constructed; note that all the primers are localized into the intronic regions.

2.4.2. PAX_2

Fragments spanning exons 1–12 of the PAX_2 gene were amplified from genomic DNA using PCR primers in the introns flanking the exons [14], as previously described [15]. Promoter was divided into three overlapping parts of about 300 bp [14] and the following primer sequences were used to amplify:

P1F 5' GGG CTC CAG CGC TGG CGA ATC ACA 3'
P1R 5' GAG AAG TAG CAA TCC CGG GG-3'
P2F 5' CTG GCC CGC GCG CTC CCC TC-3'
P2R 5' TGG GCG GTC AGC AGA ATG GC-3'
P3F 5' CGA GCC ATG CGC CCC CAG TG-3'
P3R 5' CGC CGC CGT GCC TCT CAA ACT 3'

The PCR products were purified and directly sequenced using the big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystem, Foster City, CA) and an ABI PRISM 310 sequencer (Perkin Elmer, USA). PAX_2 mutations could include a deletion of either a part or the whole PAX_2 gene from one allele. Such deletions may not necessarily be detected by sequencing. To investigate whether deletions of the whole PAX_2 gene occurred, heterozygosity of the PAX_2 gene was analyzed in each patient by amplification of a (CA)n nucleotide repeat within intron 9 as previously reported [14]. Amplification of both alleles demonstrated heterozygosity to (CA)n dinucleotide repeat. No polymorphisms and mutations of the PAX_2 gene were detected in any of the subjects.

3. Discussion

It is common clinical experience that most cases of renal agenesis and dysplasia are sporadic [2]. However, in 1974, Cain et al. [16] firstly reported 12 pedigrees with familial renal malformations including unilateral renal agenesis and described a kindred with two siblings.

There is considerable anecdotal evidence that congenital abnormalities represent the results of breakdown in several steps that regulate renal development [17]. Failure in anyone step of the nephrogenesis can be associated with urogenital malformations that include renal agenesis and malignancies [18]. In fact, it has been demonstrated that nephrogenesis is controlled by several essential molecules (transcription factors, growth factors and adhesion molecules), some of which may act as inductive signals [17]. PAX_2 and EMX_2 genes play a critical role in human kidney development [17].

The mouse homeobox gene EMX_2 is required for kidney development given that homozygous mutant mice entirely lack kidneys, ureters and gonads [8]. Moreover, it is expressed in the ureteric bud that undergoes rapid degeneration after contact with the metanephric mesenchyme in the EMX_2-/- homozygote with loss of PAX_2, LIM_1 and c-ret expression. The loss of the ureteric bud is not due to the absence of glial derived neurotrophic factor (GDNF) since this factor is present even in EMX_2 mutant mesenchymes and the initial stage of GDNF-induced outgrowth from the mesonephric duct occurs normally in these mutants. This supports the hypothesis that the EMX_2 mutant ureteric bud may lose the ability to respond to GDNF [8]. In fact, a loss of c-ret expression in the tip of the ureteric bud has been observed in the EMX_2-/- homozygotes after it has invaded metanephric blastema [8]. In addition, EMX_2 is intensely expressed in epithelial components of pronephros and mesonephros, in Wolffian and Mullerian ducts, in the ureteric bud, in the early epithelial structures derived from metanephric mesenchyme and in bipotential or indifferent gonads and ovaries [19]. These data show that EMX_2 plays a crucial role in the morphogenesis of the urogenital system.

The PAX_2 gene is a member of the paired-box class of transcription factors that plays an important role in urinary tract development [20]. In fact, homozygous PAX_2 knockout mice do not survive embryonic development and fail to develop a urogenital system [21–23].

Since the EMX_2 and PAX_2 genes are not responsible for URA in these families and many other genes (Eya 1, Gndf and RET) [24] may be involved in nephrogenesis [25], non-allelic heterogeneity might be responsible for the various cases of familial URA.

References

[1] B. Doray, B. Gasser, I. Reinartz, C. Stoll, Hereditary renal adysplasia in a three generations family, Genet. Couns. 10 (1999) 251–257.

[2] V. Duke, R. Quinton, I. Gordon, P.M.G. Bouloux, A.S. Woolf, Proteinuria, hypertension and chronic renal failure in X-linked Kallmann's syndrome, a defined genetic cause of solitary functioning kidney, Nephrol. Dial. Transplant. 13 (1998) 1998–2003.

[3] G. Corsello, E. Maresi, A.M. Corrao, U. Dimita, M. Lo Cascio, M. Cammarata, L. Giuffrè, VATER/VACTERL association: clinical variability and expanding phenotype including laryngeal stenosis, Am. J. Med. Genet. 47 (1992) 813–815.

[4] W.L. Robson, A.K. Leung, R.C. Rogers, Unilateral renal agenesis, Adv. Pediatr. 42 (1995) 575–592.

[5] M. Suri, L.A. Brueton, N. Venkatraman, P.M. Cox, MURCS association with encephalocele: report of a second case, Clin. Dysmorph. 9 (2000) 31–33.

[6] Y. Legros, J.R. Carrenard, F.M. Cortes, J.M. Sanchez, Sindrome de "ojo de gato" con agenesia renal derecha. Aportacion de un caso y revision de la literatura, An. Esp. Pediatr. 39 (1992) 317–319.

[7] V.A. McKusick, Mendelian Inheritance in Man, 10th ed, Johns Hopkins University Press, Baltimore, 1992, pp. 1131–1132.

[8] N. Miyamato, M. Yoshida, S. Kuratani, I. Matsuo, S. Aizawa, Defects of urogenital development in mice lacking EMX_2, Development 124 (1997) 1653–1664.

[9] P. Sanyausin, L.A. McNoe, N. Sullivan, R.G. Weaver, M.R. Eccles, Mutation of PAX_2 in two siblings with renal-coloboma syndrome, Hum. Mol. Genet. 4 (1995) 2183–2184.

[10] E. Torban, P. Goodyer, What PAX genes do in the kidney, Exp. Nephrol. 6 (1998) 7–11.

[11] T. Ogata, K. Muroya, I. Sasagawa, T. Kosho, K. Wakui, S. Sakazume, K. Ito, N. Matsuo, H. Ohashi, T. Nagai, Genetic evidence for a novel gene(s) involved in urogenital development on 10q26, Kidney Int. 58 (2000) 2281–2290.

[12] K. Nishimoto, K. Iijma, T. Shirakawa, K. Kitagawa, K. Satomura, H. Nakamura, N. Yoshikawa, *PAX*$_2$ gene mutation in a family with isolated renal hypoplasia, J. Am. Soc. Nephrol. 12 (2001) 1769–1772.
[13] S. Brunelli, A. Faiella, V. Capra, V. Nigro, A. Simeone, A. Cama, E. Boncinelli, Germline mutations in the homeobox gene *EMX*$_2$ in patients with severe schizencephaly, Nature Genet. 12 (1996) 94–96.
[14] P. Sanyanusin, J.H. Norrish, T.A. Ward, A. Nebel, L.A. McNoe, M.R. Eccles, Genomic structure of the human *PAX*$_2$ gene, Genomics 35 (1996) 258–261.
[15] L.A. Schimmenti, H.E. Cunliffe, L.A. McNoe, T.A. Ward, Y.H. Zhang, W. Proesmans, A. Leys, K.A. Byerly, S.R. Braddock, M. Masuno, K. Imaizumi, K. Devtriendt, M.R. Eccles, Further delineation of renal-coloboma syndrome in patients with extreme variability of phenotype and identical PAX$_2$ mutations, Am. J. Hum. Genet. 60 (1997) 869–878.
[16] D.R. Cain, D. Griggs, D.A. Lackey, B.M. Kagan, Familial renal agenesis and total dysplasia, Am. J. Dis. Child 128 (1974) 377–380.
[17] A.S. Woolf, Embriology of the kidney, in: T.M. Barratt, A. Avner, W. Harmon (Eds.), Pediatric Nephrology, 4th ed, Williams & Wilkins, Baltimore, 1999, pp. 1–19.
[18] E.L. Potter, Bilateral renal agenesis, J. Pediatr. 29 (1946) 68–76.
[19] M. Pellegrini, S. Pantano, F. Lucchini, M. Fumi, A. Forabosco, EMX$_2$ developmental expression in the primordia of the reproductive and excretory systems, Anat. Embryol. 196 (1997) 427–433.
[20] A. Mausoury, M. Hallonet, P. Gruss, *PAX* genes and their roles in cell differentiation and development, Curr. Opin. Cell. Biol. 8 (1996) 851–857.
[21] M. Torres, E. Gomez-Pardo, G. Dressler, P. Gruss, PAX$_2$ controls multiple steps of urogenital development, Development 121 (1995) 4057–4065.
[22] J. Favor, R. Sandulache, A. Neuhauser-Klaus, W. Pretsch, B. Chatterjee, E. Senft, W. Wurst, V. Blanquet, P. Grimes, R. Sporle, K. Schughart, The mouse PAX$_2$^{1Neu} mutation is identical to a human PAX$_2$ mutation in a family with renal coloboma syndrome and results in development defects of the brain, ear, eye, and kidney, Proc. Natl. Acad. Sci. USA 93 (1996) 13870–13875.
[23] H.E. Cunliffe, L.A. McNoe, T.A. Ward, K. Devriendt, H.G. Brunner, M.R. Eccles, The prevalence of PAX$_2$ mutations in patients with isolated colobomas or colobomas associated with urogenital anomalies, J. Med. Genet. 35 (1998) 806–812.
[24] T.D. Piscione, N.D. Rosenblum, The malformed kidney: disruption of glomerular and tubular development, Clin. Genet. 56 (1999) 341–356.
[25] C.R. Burrow, Regulatory molecules in kidney development, Pediatr. Nephrol. 14 (2000) 240–253.