J Am Soc Nephrol 12: 1769–1772, 2001

PAX2 Gene Mutation in a Family with Isolated Renal Hypoplasia

KAORI NISHIMOTO,* KAZUMOTO IIJIMA,† TAKU SHIRAKAWA,* KOUSAKU KITAGAWA,‡ KENICHI SATOMURA,§ HAJIME NAKAMURA,† and NORISHIGE YOSHIKAWA‖

**Faculty of Health Science and †Department of Pediatrics, Kobe University School of Medicine, Kobe, Japan; ‡Department of Pediatrics, Takasago Municipal Hospital, Takasago, Japan; §Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan; and ‖Department of Pediatrics, Wakayama Medical University, Wakayama, Japan.*

Abstract. The *PAX2* gene encodes a transcription factor that plays a critical role in the development of the urogenital tract, eyes, ears, and central nervous system. Recently, renal hypoplasia was observed to be part of the renal-coloboma syndrome, which is caused by heterozygous mutations of the *PAX2* gene. The renal-coloboma syndrome is a rare autosomal dominant syndrome that involves optic nerve colobomas and renal anomalies. For investigation of whether *PAX2* mutations occur in patients with isolated renal hypoplasia, patient DNA was analyzed for *PAX2* mutations, by using PCR and direct sequencing. The study involved 20 patients with bilateral renal hypoplasia associated with decreased renal function. Heterozygous *PAX2* mutations were detected in two patients, *i.e.*, a novel nonsense mutation (C to A transversion at position 1566 in exon 9) in patient 1 and another novel nonsense mutation (C to T transversion at position 1318 in exon 7) in patient 2. The nucleotide changes for patients 1 and 2 directly introduced stop codons, presumably resulting in a message for a truncated PAX2 protein that lacked a partial transactivation domain. An ophthalmologic examination revealed a very mild, asymptomatic coloboma in patient 2, whereas the fundus was normal for patient 1. The mutation cosegregated with the presence of renal hypoplasia in the family of patient 1, appearing *de novo* in the mother of the patient, which strongly suggests that this mutation was the cause of renal hypoplasia in this family. This study demonstrates for the first time that *PAX2* mutations can be responsible for isolated renal hypoplasia.

Renal hypoplasia is a common childhood condition characterized by a reduction in the number of nephrons and a small overall kidney size. Bilateral hypoplasia is a major cause of end-stage renal failure among children and is an important cause among adults. Recently, renal hypoplasia was observed in the renal-coloboma syndrome, which is caused by mutations of the *PAX2* gene (1–3). The renal-coloboma syndrome is a rare autosomal dominant syndrome involving optic nerve colobomas and renal anomalies. *PAX2* encodes a transcription factor that plays a critical role in the development of the urogenital tract, eyes, ears, and central nervous system (4). Homozygous *PAX2* mutant mice lack kidneys, ureters, and a genital tract, whereas heterozygous mutant mice frequently develop hypoplastic kidneys (5).

Because phenotypic variability is a feature of the renal-coloboma syndrome and renal hypoplasia is the most common congenital renal anomaly in humans with *PAX2* gene mutations, we hypothesized that *PAX2* mutations may account for some cases of isolated renal hypoplasia. However, the specific genes responsible for causing isolated renal hypoplasia are not known. To investigate whether *PAX2* mutations occur in patients with isolated renal hypoplasia, we analyzed patient DNA for *PAX2* mutations, using PCR and direct sequencing, for 20 patients with bilateral renal hypoplasia associated with decreased renal function. This study demonstrated for the first time that *PAX2* mutations could be responsible for isolated renal hypoplasia.

Materials and Methods

Patients

Twenty unrelated Japanese patients (12 male and 8 female patients) with bilateral hypoplastic kidneys were examined after informed consent was obtained. In this study, we clinically defined a hypoplastic kidney as a small kidney. All patients demonstrated decreased renal function (creatinine clearance, <50 ml/min per 1.73 m^2); eight patients were undergoing regular dialysis therapy and two patients had undergone renal transplantation. All patients were examined by expert ophthalmologists, and all had normal eyesight. Cystograms were obtained for all patients and revealed vesicoureteral reflux for four patients. Renal biopsies were performed for 11 patients, 10 of whom exhibited oligomeganephronic renal hypoplasia (defined on the basis of reduced numbers of nephrons and nephron hypertrophy) (Figure 1) and one of whom exhibited simple renal hypoplasia. Three patients demonstrated family histories of renal hypoplasia.

Received July 7, 2000. Accepted February 14, 2001.

Correspondence to Dr. Norishige Yoshikawa, Department of Pediatrics, Wakayama Medical University, 811-1 Kimiidera, Wakayama City, Japan 641-8510. Phone: +81-73-441-0632; Fax: +81-73-444-9055; E-mail: nori@wakayama-med.ac.jp

1046-6673/1208-1769
Journal of the American Society of Nephrology

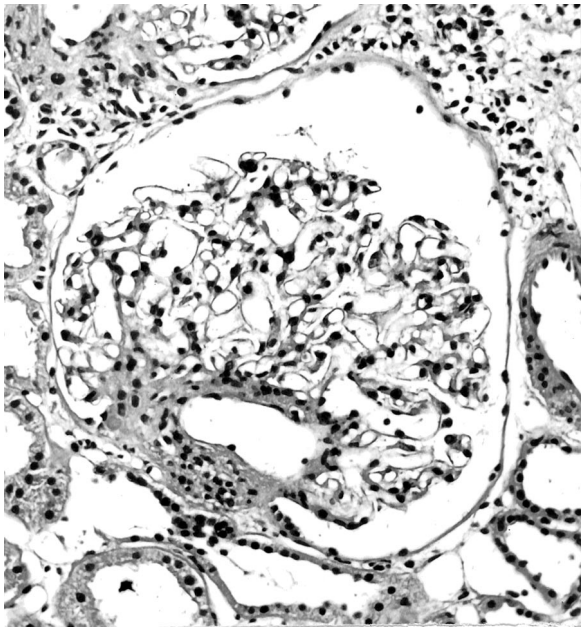

Figure 1. Renal biopsy for patient 18 at 5 yr of age. A glomerulus is markedly enlarged. Magnification, ×200 (hematoxylin and eosin).

DNA Sequencing

Genomic DNA was extracted and purified from peripheral leukocytes in whole-blood samples by using a SepaGene kit (Sanko, Tokyo, Japan). All primers were designed from intronic sequences (Table 1). The PCR products were purified by using a QIAquick PCR purification kit (Qiagen, Hilden, Germany) and PCR-amplified by using a dye-terminator, cycle-sequencing, BD Ready Reaction kit (Perkin Elmer, Branchburg, NJ). The cycle-sequencing product was then analyzed by using an automated sequencer (ABI PRISM 310 genetic analyzer or ABI PRISM 377 automated sequencer; Perkin Elmer, Foster, CA).

Results

Heterozygous *PAX2* mutations were detected for two patients. We observed two heterozygous, novel, nonsense *PAX2* mutations in these two patients. A careful ophthalmologic examination revealed a very mild, asymptomatic coloboma for one patient, whereas the fundus was normal for the other patient. Two single-nucleotide polymorphisms (1410C→T and 1521A→C) in the coding region were observed for seven patients (6).

Patient 1

Patient 1 was an 8-yr-old Japanese boy (Figure 2). He was delivered by caesarean section at 38 wk of gestation, and his birth weight was 2655 g. At the age of 3 mo, his weight gain was poor. His serum creatinine concentration was 1.3 mg/dl, and his blood urea nitrogen level was 26 mg/dl. Renal ultrasonography demonstrated bilateral small kidneys. At 7 yr of age, a renal investigation revealed renal insufficiency, bilateral small kidneys (right, 56 mm; left, 53 mm; normal size for age, 79 ± 2.5 mm), and bilateral grade III vesicoureteral reflux. Further investigations at 8 yr of age demonstrated progressive renal failure (serum creatinine concentration, 2.8 mg/dl; blood urea nitrogen level, 61 mg/dl). An eye examination demonstrated normal visual acuity and a normal optic nerve. The physical examination revealed normal growth, intelligence, and hearing. A renal biopsy was not performed.

The 27-yr-old mother of the patient also had bilateral renal hypoplasia. She had exhibited proteinuria since the age of 3 yr. End-stage renal disease and bilateral renal hypoplasia had been diagnosed at the age of 8 yr, and the subject was being maintained on hemodialysis while awaiting a second renal transplant, after the failure of her first transplant. No eye or external ear anomalies or hearing losses were present, and the subject had normal intelligence.

A nonsense mutation, namely a C to A substitution at position 1566 in exon 9, was identified for both patient 1 and his mother. This nucleotide change directly introduced a stop codon (TAA), presumably resulting in a message for a truncated PAX2 molecule. This mutation was not observed for the father, maternal aunt, or maternal grandparents of the patient. His father, maternal aunt, and maternal grandparents exhibited normal kidneys in ultrasound examinations, normal renal function, and normal eyes.

Table 1. PAX2 genomic DNA PCR primer list

Exon	Sense Primer	Antisense Primer	Product Length (bp)	Annealing Temperature (°C)
1	GTTCACTCATCCTCCCTCCCCCACC	GGAGCCGGGCGCGGGTACTC	179	62
2	CTGTGTGTGGGGTGTTGTGTT	AAGGCGTCTTCCCGGGACAGCTGC	246	64
3	TGACCGGCTTTCCCGGCGCA	GAGGAAGCTGGAGTCCAGCC	262	62
4	CGGAATAGGAGTGGCATTTGA	CTCTAGGTGGGATCTGGTTT	181	58
5	TGATGCCATTTCCTCCTTCC	GCCACACCTCTTCCCTCCT	175	60
7	CGCCCCGAGTGTCCATGTGTT	TACTTCTGCAAGCAGAAAGCTCCCT	234	58
8	CGTGCATCAATAGAGAGCTGTCAC	AGCCCCCTTACCAGTCACAAC	180	59
9	CCCTTCCCCTTTGTGTTTTT	AGGCAGCTGCAGCATTGTC	151	60
11, 1st	GCAGGCGTCACATCCC	ATGTGGAGGCCGAAGCTGT		57
11, 2nd	GCAGGCGTCACATCCC	CCAGGTGGCATACTCACTTA	141	57
12	TCTGACCCAGCCCATTCTTCT	ATGTGGAGGCCGAAGCTGT	163	58

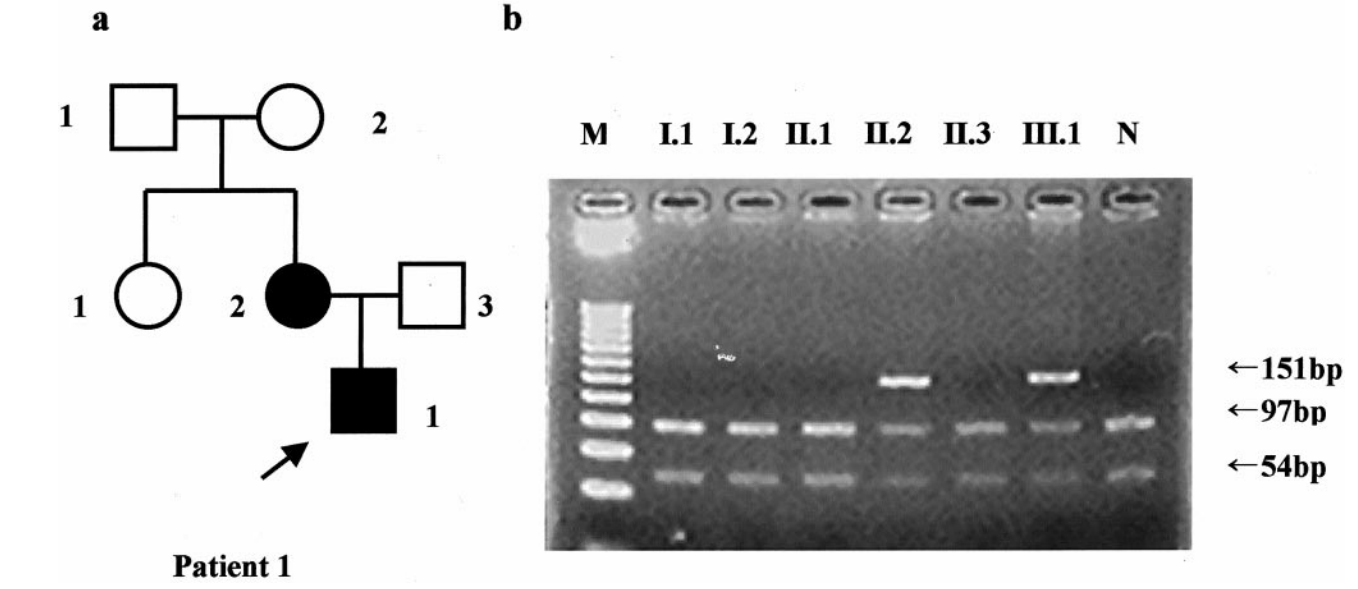

Figure 2. Pedigree of and *PAX2* mutation in the family of patient 1. (A) Pedigree of the family of patient 1. □, unaffected male subjects; ■, male subject with renal hypoplasia; ○, unaffected female subjects; ●, female subject with renal hypoplasia. (B) *Bst*EII restriction pattern of PCR-amplified exon 9 of DNA samples. DNA sequence analysis for patient 1 revealed a heterozygous C to A transversion at position 1566. This mutation resulted in the lack of a *Bst*EII site. PCR amplification and *Bst*EII digestion resulted in two products (97 and 54 bp) for the normal sequence (N) but three products (151, 97, and 54 bp) for the mutant sequence. Patient 1 (III.1) and his mother (II.2) are heterozygous for the wild-type and mutant alleles. M, 25-bp DNA marker.

Patient 2

Patient 2 was a 4-yr-old Japanese girl (Figure 3). She was delivered at 40 wk of gestation after an uncomplicated pregnancy, and her birth weight was 2560 g. At the age of 1 mo, her weight gain was poor, her serum creatinine concentration was 0.7 mg/dl, and her blood urea nitrogen level was 61 mg/dl. Renal ultrasonography demonstrated bilateral small kidneys. At 3 yr of age, a renal investigation revealed renal insufficiency, bilateral small kidneys (right, 55 mm; left, 52 mm; normal size for age, 70 ± 1.7 mm), and no vesicoureteral reflux. Recent investigations conducted at 4 yr of age demonstrated chronic renal insufficiency (serum creatinine concentration, 0.5 mg/dl; blood urea nitrogen level, 27 mg/dl). Careful ophthalmoscopy revealed very mild, asymptomatic, optic nerve atrophy in the right eye and a normal optic nerve in the left eye. Visual acuity was normal. The physical examination revealed normal growth, intelligence, and hearing. A renal biopsy was not performed.

A nonsense mutation, namely a C to T substitution at position 1318 in exon 7, was identified for patient 2. This nucleotide change directly introduced a stop codon (TAG), presumably resulting in a message for a truncated PAX2 molecule. This mutation was not observed in the parents of the patient or her two siblings. Her parents and siblings exhibited normal kidneys in ultrasound examinations, normal renal function, and normal eyes.

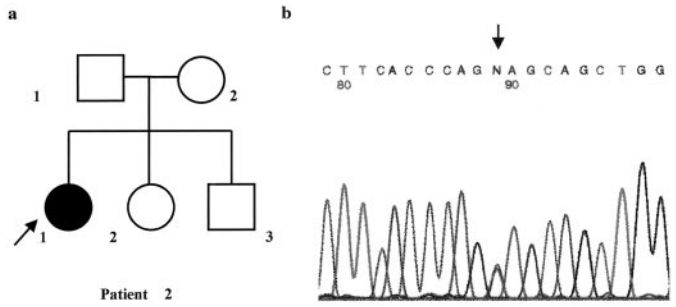

Figure 3. Pedigree of the family of patient 2 and *PAX2* mutation in patient 2. (A) Pedigree of the family of patient 2. □, unaffected male subjects; ○, unaffected female subjects; ●, female subject with renal-coloboma syndrome (patient 2). (B) Mutation in patient 2. DNA sequence analysis for patient 2 revealed a heterozygous C to T transversion at position 1318 in exon 7. This nucleotide change directly introduced a stop codon (TAG).

Discussion

Two novel nonsense *PAX2* mutations were identified in two of 20 patients with bilateral small kidneys and renal insufficiency. All previously reported patients with *PAX2* mutations exhibited optic nerve colobomas (1,2,7–11). A careful ophthalmologic examination demonstrated an optic nerve coloboma for patient 2 but not patient 1. The nucleotide change directly introduced a stop codon (TAA), presumably resulting in a message for a truncated PAX2 molecule. The mutation cosegregated with the presence of renal hypoplasia in the family of patient 1 and appeared *de novo* in his mother, strongly suggesting that this mutation was the cause of the renal hypoplasia in this family. Thus, this study has demonstrated for the first time that *PAX2* mutations can be responsible for isolated renal hypoplasia.

The *PAX2* gene resides on human chromosome 10 (8) and consists of 12 exons (4). Exons 1 to 4 include the paired box domain. Exon 5 contains another highly conserved motif, *i.e.*, the octapeptide sequence, whose function is not clear. The carboxy-terminal portion of the PAX2 protein, encoded by exons 7 to 12, is thought to be important for transcriptional activation of target genes by the PAX2 protein (12).

All except one of the previously described mutations in *PAX2* occurred within the conserved paired box and octapeptide sequences contained in exons 1 to 5 (1,2,7–11). The majority of *PAX2* mutations lead to a truncated protein, probably resulting in target loss. The two mutations described here are unique, in that they are located in exons 7 and 9, which encode a partial homeodomain. The precise effects of these two mutations are not known, but it is thought that they probably lead to disruptions in the structure of these portions of the PAX2 protein and loss of normal function. The abnormal PAX2 proteins in patients 1 and 2 should still be able to bind DNA, because the paired box domain would remain intact, but they may lack the ability to transactivate the expression of target genes. PAX2 is thought to function as a transcription factor, probably regulating the expression of one or more of the critical genes involved in kidney differentiation.

Specific *PAX2* mutations have not been associated with specific phenotypic features (7). In this study, patient 1 and his mother demonstrated a novel C to A substitution at position 1566 of *PAX2*, which resulted in a change from a tyrosine codon to a stop codon in exon 9. This mutation is the most 3' mutation identified in the *PAX2* gene to date. The protein resulting from this mutant allele is predicted to be truncated midway through the partial homeodomain, which would result in loss of the partial transactivation domain. However, patient 1 and his mother exhibited no eye manifestations. Therefore, lack of the partial transactivation domain of *PAX2* may not lead to eye anomalies.

Renal hypoplasia is the most common renal anomaly in humans and mice with *PAX2* gene mutations (11). Although it is clear that *PAX2* plays a critical role during kidney development, the precise pathogenesis of renal hypoplasia resulting from *PAX2* mutations is not known. Porteous *et al.* (11) recently suggested that heterozygous mutations of *PAX2* are associated with increased apoptosis and reduced branching of the ureteric bud, because of a reduction in *PAX2* gene dosage during a critical period in kidney development. We detected no *PAX2* mutations among patients with biopsy-proven oligomeganephronic renal hypoplasia. Patients 1 and 2 did not undergo renal biopsies. *PAX2* mutations were reported for patients with renal-coloboma syndrome and oligomeganephronic renal hypoplasia (13). Recently, a hepatocyte nuclear factor-1β gene mutation was observed for a patient with diabetes mellitus and biopsy-proven oligomeganephronic renal hypoplasia (14). In conclusion, this study demonstrates for the first time that *PAX2* mutations can be responsible for isolated renal hypoplasia, and it suggests that isolated renal hypoplasia in patients with *PAX2* mutations is part of the spectrum of the renal-coloboma syndrome.

References

1. Sanyanusin P, Schimmenti LA, McNoe LA, Ward TA, Pierpont ME, Sullivan MJ, Dobyns WB, Eccles MR: Mutation of the PAX2 gene in a family with optic nerve colobomas, renal anomalies and vesicoureteral reflux. *Nat Genet* 9: 358–364, 1995
2. Sanyanusin P, McNoe LA, Sullivan MJ, Weaver RG, Eccles MR: Mutation of PAX2 in two siblings with renal-coloboma syndrome. *Hum Mol Genet* 4: 2183–2184, 1995
3. Schimmenti LA, Pierpont ME, Carpenter BL, Kashtan CE, Johnson MR, Dobyns WB: Autosomal dominant optic nerve colobomas, vesicoureteral reflux, and renal anomalies. *Am J Med Genet* 59: 204–208, 1995
4. Sanyanusin P, Norrish JH, Ward TA, Nebel A, McNoe LA, Eccles MR: Genomic structure of the human PAX2 gene. *Genomics* 35: 258–261, 1996
5. Torres M, Gomez Pardo E, Dressler G, Gruss P: Pax2 controls multiple steps of urogenital development. *Development* 121: 4057–4065, 1995
6. Shim HH, Nakamura BN, Cantor RM, Schimmenti LA: Identification of two single nucleotide polymorphisms in exon 8 of PAX2. *Mol Genet Metab* 68: 507–510, 1999
7. Schimmenti LA, Cunliffe HE, McNoe LA, Ward TA, French MC, Shim HH, Zhang YH, Proesmans W, Leys A, Byerly KA, Braddock SR, Masuno M, Imaizumi K, Devriendt K, Eccles MR: Further delineation of renal-coloboma syndrome in patients with extreme variability of phenotype and identical PAX2 mutations. *Am J Hum Genet* 60: 869–878, 1997
8. Narahara K, Baker E, Ito S, Yokoyama Y, Yu S, Hewitt D, Sutherland GR, Eccles MR, Richards RI: Localisation of a 10q breakpoint within the PAX2 gene in a patient with a de novo t(10;13) translocation and optic nerve coloboma-renal disease. *J Med Genet* 34: 213–216, 1997
9. Devriendt K, Matthijs G, Van Damme B, Van Caesbroeck D, Eccles M, Vanrenterghem Y, Fryns JP, Leys A: Missense mutation and hexanucleotide duplication in the PAX2 gene in two unrelated families with renal-coloboma syndrome (MIM 120330). *Hum Genet* 103: 149–153, 1998
10. Cunliffe HE, McNoe LA, Ward TA, Devriendt K, Brunner HG, Eccles MR: The prevalence of PAX2 mutations in patients with isolated colobomas or colobomas associated with urogenital anomalies. *J Med Genet* 35: 806–812, 1998
11. Porteous S, Torban E, Cho NP, Cunliffe H, Chua L, McNoe L, Ward T, Souza C, Gus P, Giugliani R, Sato T, Yun K, Favor J, Sicotte M, Goodyer P, Eccles M: Primary renal hypoplasia in humans and mice with PAX2 mutations: Evidence of increased apoptosis in fetal kidneys of $Pax2^{1Neu}$ +/− mutant mice. *Hum Mol Genet* 9: 1–11, 2000
12. Lechner MS, Dressler GR: Mapping of Pax-2 transcription activation domains. *J Biol Chem* 271: 21088–21093, 1996
13. Salomon R, Attie T, Tellier AL, Antignac C, Lyonnet S, Vekemans M, Munnich A, Dureau P, Broyer M, Gubler MC, Niaudet P: PAX-2 mutations in oligomeganephronic renal hypoplasia. *J Am Soc Nephrol* 10: 442A, 1999
14. Lindner TH, Njolstad PR, Horikawa Y, Bostad L, Bell GI, Sovik O: A novel syndrome of diabetes mellitus, renal dysfunction and genital malformation associated with a partial deletion of the pseudo-POU domain of hepatocyte nuclear factor-1β. *Hum Mol Genet* 8: 2001–2008, 1999