Multicystic Dysplastic Kidney and Variable Phenotype in a Family with a Novel Deletion Mutation of *PAX2*

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The renal coloboma syndrome (OMIM 120330) is caused by mutations in the *PAX2* gene. Typical findings in these patients include renal hypoplasia, renal insufficiency, vesicoureteric reflux, and optic disc coloboma. A family with a novel heterozygous 10-bp deletion in exon 2 of the *PAX2* gene leading to a truncating mutation and variable phenotype across three generations is reported. The first presentation of multicystic dysplastic kidney in this syndrome is reported. The possibility that abnormal *PAX2* protein in this case may cause a dominant negative effect also is discussed. The finding of multicystic dysplastic kidney in renal coloboma syndrome could suggest that *PAX2* may play a role in early ureteric obstruction and subsequent renal maldevelopment.

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Renal coloboma syndrome is an autosomal dominant disorder, characterized by renal malformations and optic disc coloboma. Renal and/or ocular anomalies are found in all patients, but the type and the severity of these abnormalities can vary markedly. Commonly observed manifestations that affect the kidneys include renal hypoplasia and vesicoureteric reflux (VUR), both of which can lead to renal insufficiency (1–9). Extrarenal manifestations can include sensorineural hearing loss, Arnold Chiari malformation, seizures of unknown cause, and joint laxity, but these are reported in <20% of patients (1,5,7,10).

Heterozygous mutations in the human *PAX2* gene were first described in 1995 in patients with the renal coloboma syndrome (4,8). Studies with *Pax2*-knockout mice have revealed that homozygous deletions lead to early postnatal death with absence of the kidneys, ureters, and eyes (11,12). The human *PAX2* gene is located on chromosome 10q24-25. It comprises 12 exons, with exons 2, 3, and 4 encoding a highly conserved 128–amino acid DNA binding domain (2,13). *PAX2* encodes a transcription factor that belongs to the "paired-box" family of homeotic genes. During embryogenic development, it is abundantly expressed in the kidney, eye, cochlear (14), pancreas (15), and central nervous system (11,16,17) and is involved in the regulation of several genes, such as *WT1* (18), *N-myc* (19), and *p53* (19).

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PAX2 mutations that cause renal coloboma syndrome have been identified in the exons encoding the DNA binding domain (exons 2 and 3), the octapeptide domain (exon 5), the homeodomain (exon 7), and the transactivation domain (exons 8 and 9). All mutations (Table 1) are recorded in the human *PAX2* allelic variant database (http://pax2.hgu.mrc.ac.uk/) and include nucleotide substitutions (4,6,20,21), insertions (1,6,7,9,10), and deletions (1,5,7,10,22–24), with a single reported case of a *de novo* translocation (3,10,13). Although variability is found with differing mutations, there is also phenotypic variability with the same mutation and within the same family. *PAX2* mutations have been reported in one patient with isolated renal hypoplasia (20) and in patients with isolated VUR (25,26). No patients with isolated ocular coloboma have been found thus far to have *PAX2* mutations (22).

Because of the commonly associated finding of ureteric atresia, multicystic dysplastic kidney (MCDK) is thought to arise from congenital ureteral obstruction during early nephrogenesis (27). Some forms of unilateral renal agenesis could also be due to the involution of early MCDK (27). Anatomic and structural studies have suggested that the anomalies observed in MCDK arise from malformation of the ureteric bud branches and ampullae (28). MCDK has not previously been reported in patients with renal coloboma syndrome; however, unilateral renal agenesis has been reported in two patients and is observed in 1 to 6% of mice with the $Pax2^{1Neu}$ mutation (1,12,21,29). Renal agenesis, unilateral kidney cysts, dilated ureters, and cystic changes within the renal medulla have also been observed in small numbers of Kidney and Retinal Defects (*Krd*) transgenic mice (12).

We now describe a family who have renal coloboma syndrome and a novel heterozygous 10-bp deletion in exon 2 of *PAX2*, which leads to a shift in the open reading frame and thus a chimeric protein that comprises *PAX2* residues 1 to 46 fol-

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Table 1. Reported *PAX2* mutations in the human *PAX2* allelic variant database (http://pax2.hgu.mrc.ac.uk/) and clinical phenotype associated with each mutation[a]

Mutation Type	Mutation	Location (Nucleotide)	Renal Phenotype	Ocular Phenotype	Other	Reference
Substitution	n.769G>A Missense	Exon 3 (769)	Mild renal impairment	Mild eye anomalies		6
	n.1228C>T Nonsense	Exon 7 (1228)	Bilateral renal hypoplasia	Bilateral optic nerve coloboma		21
	n.1249C>T Nonsense	Exon 7 (1249)	Bilateral renal hypoplasia; chronic renal insufficiency	Asymptomatic right optic nerve atrophy		20
	n.1497C>A Nonsense	Exon 9 (1497)	Isolated renal hypoplasia; neonatal renal insufficiency; bilateral small kidneys; bilateral vesicoureteric reflux	Normal		20
Insertions	n.619insG Frame shift	Exon 2 (619)	Renal tubular atrophy; interstitial fibrosis; proteinuria; end-stage renal failure	Bilateral optic nerve coloboma		4
	n.619insG Frame shift	Exon 2 (619)	Bilateral renal hypoplasia; end-stage renal failure; focal segmental glomerulosclerosis	Bilateral optic nerve coloboma	Right high-frequency hearing loss; joint laxity	10
	n.619insG Frame shift	Exon 2 (619)	Bilateral renal hypoplasia; end-stage renal failure	Left optic nerve aplasia; right optic disc hypoplasia with optic pit; chorioretinal coloboma; capsular opacities; microphthalmos; retrobulbar cyst	Retardation; microcephaly	10
	n.619insG Frame shift	Exon 2 (619)	Bilateral renal hypoplasia	Bilateral microphthalmia; right optic nerve dysplasia		1
	n.619insG Frame shift	Exon 2 (619)	Bilateral renal hypoplasia	Bilateral optic nerve coloboma		1
	n.619insG Frame shift	Exon 2 (619)	Bilateral renal hypoplasia	Morning glory syndrome		1
	n.619insG Frame shift	Exon 2 (619)	Oligomeganephronia; papillary dysplasia	Nil		24
	n.619insGG Frame shift	Exon 2 (619)	Bilateral renal hypoplasia	Bilateral optic nerve coloboma		1
	n.619insG Frame shift	Exon 2 (619)	Bilateral renal hypoplasia	Bilateral abnormal optic discs; left macular hypoplasia; right morning glory syndrome		9
	n.619insG Frame shift	Exon 2 (619)	Oligomeganephronia	Bilateral optic nerve coloboma		24
	n.619insG Frame shift	Exon 2 (619)	Chronic renal insufficiency	Bilateral optic nerve coloboma; myopia	Arnold Chiari type I malformation	7
	n.619insG Frame shift	Exon 2 (619)	Small kidneys; progressive renal failure	Bilateral optic nerve coloboma with retinal detachment	Bilateral cryptorchidism	21
	n.768–769ins6 In-frame	Exon 3 (768)	Mild renal dysfunction; VUR	Severe visual impairment		6
Deletions	n.602delT Frame shift	Exon 2 (602)	Bilateral renal hypoplasia	Bilateral optic nerve coloboma	Rickets; umbilical and right inguinal hernia	23
	n.611delT Frame shift	Exon 2 (611)	Bilateral renal hypoplasia; left kidney VUR	Bilateral optic nerve coloboma		22
	n.619delG Frame shift	Exon 2 (619)	Bilateral optic nerve coloboma; VUR	Nystagmus; esotropia	fourth and fifth digit clinodactyly	7
	n.658–663del6 In-frame	Exon 2 (658)	Oligomeganephronia	Bilateral optic nerve coloboma		24
	n.673–694del22 Frame shift	Exon 2 (673)	Bilateral renal hypoplasia; proteinuria; progressive end-stage renal failure	Bilateral optic nerve coloboma	Seizures	10
	n.832delG Frame shift	Exon 3 (832)	Unilateral renal agenesis	Bilateral optic nerve coloboma; severe myopia		1
	n.1104delC Frame shift	Exon 5 (1104)	Bilateral renal hypoplasia; nonfunctional right kidney; renal failure; grade IV VUR	Bilateral optic nerve coloboma	Joint laxity	5
Translocation	De novo translocation	Chr 10; Chr 13	Renal tubular atrophy; glomerulosclerosis; mild renal dysfunction with proteinuria.	Bilateral optic nerve coloboma; cataracts		3

[a]VUR, vesicoureteral reflux.

lowed by 31 residues of altered amino acid sequence. The affected members of this family exhibit a variable clinical phenotype, including MCDK.

Materials and Methods

Genomic DNA was extracted from patients' peripheral blood samples (2 to 5 ml) using the SDS-proteinase K method. DNA from patient II-3 was extracted from a Papanicolaou-smear that was stored at room temperature using the QIAamp DNA Mini Kit (Qiagen Pty Ltd, Doncaster, Australia) method.

PAX2 exon 2 was amplified from genomic DNA by PCR. Oligonucleotide primers for exon 2 were designed using the Primer 3 software of Bionavigator. The forward primer was 5′-TGTGTGTGGGGTGTTGTGTT-3′ and was FAM-labeled at the 5′ end. The reverse primer was 5′-GGAAGTAGGCAAGGCGTCTC-3′. Both primers incorporated intronic DNA flanking exon 2; the expected PCR product was 253 bp in length. Primers were purchased from Invitrogen Life Technologies (Melbourne, Australia). The PCR was performed in a final volume of 50 μl, which contained 10× Taq buffer (5 μl), 0.2 mM dNTP (4 μl), TaqDNA polymerase 0.5 U (0.4 μl), 20 ng of genomic DNA (2 μl), 0.25 μM of each primer (2 μl), and H_2O (34.6 μl). The PCR profile using three-step cycling for 35 cycles was denaturation at 95°C for 30 s,

annealing at 61°C for 30 s, and elongation at 72°C for 30 s. PCR reactions were carried out using the BioRad I cycler. Spectratyping, which shows the amount of PCR product of differing lengths using fluorescence labeling with FAM of the PCR products, was used to detect changes due to insertion or deletion in exon 2 and was performed on the ABI PRISM 310 genetic analyzer. Data were extracted and analyzed using 310 GeneScan 3.1.2 software (Applied Biosystems, Foster City, CA).

Subcloning and DNA Sequencing

PCR products were purified using a QIAquick PCR purification kit (Qiagen) and cloned into the pGEM-T Easy vector system (Promega, Madison, WI) and transformed into *Escherichia coli* JMS09 competent cells. After the bacterial cells were transformed in SOC medium, 100 μl of the suspension was plated on lysogeny broth medium that contained ampicillin (100 μg/ml), IPTG (0.5 nM), and X-Gal (80 μg/ml). Plates were incubated at 37°C overnight. Colonies with insertions of the PCR product were identified. Nucleotide sequence analysis was performed on independent bacterial colonies using the Big Dye terminator cycle sequence ready reaction kit (Applied Biosystems). The samples were sequenced using an ABI PRISM 310 genetic analyzer.

Twin Zygosity Determination

Assessment for zygosity was undertaken in patients IV-2 and IV-3. Molecular zygosity determination was performed using the PowerPlex 1.2 System (Promega).

Results

Family pedigree is shown in Figure 1.

Patient 1

Patient II-3 presented at age 21 yr with right pyelonephritis. Investigations revealed mild proteinuria (0.2 g/L) and an elevated creatinine (166 μmol/L). Intravenous pyelogram showed bilaterally small kidneys with right tubular defects and abnormal calyces, and at the time a diagnosis of right medullary sponge kidney (MSK) was made. Renal biopsy at 29 yr of age was also suggestive of MSK. She subsequently developed chronic renal impairment (serum creatinine 250 μmol/L). Ophthalmoscopy and fluorescein angiography revealed optic disc coloboma (Figure 2A). Audiometry revealed bilateral high-tone sensorineural and mild conductive hearing loss. She died at the age of 51 yr after complications from ischemic heart disease.

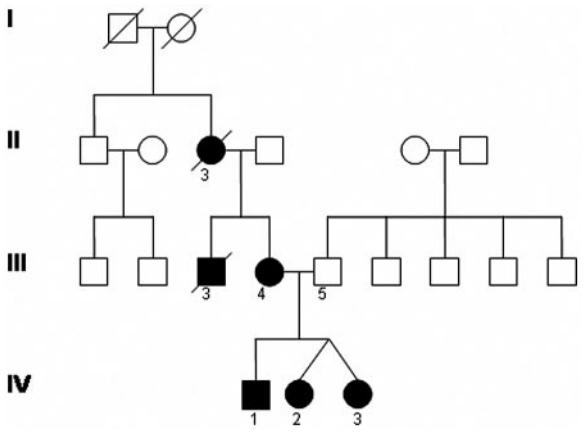

Figure 1. Pedigree of the family reported with renal coloboma syndrome showing classical autosomal dominant inheritance across three generations. □, unaffected male patients; ■, male patients with renal coloboma syndrome; ○, unaffected female patients; ●, female patients with renal coloboma syndrome; lines across affected and unaffected patients indicate deceased.

Patient 2

Patient III-3 received a diagnosis at 3 years of age of bilateral grade III to IV VUR and right secondary ureteropelvic junction (UPJ) obstruction. Renal ultrasound showed bilaterally small kidneys. An ophthalmologist's examination revealed bilateral optic disc coloboma and normal visual acuity. Proteinuria at 4 yr of age was significant at 0.8 g/L per d. Serial urinalyses using "combur 10" test strips (Roche Diagnostics, Castle Hill, New South Wales) showed persistent heavy (4+) proteinuria. He was treated for hypertension and developed left ventricular hypertrophy diagnosed by echocardiography. Renal osteodystrophy was treated with oral vitamin D supplementation. Hemodialysis was commenced at the age of 14 yr, and he died at age 16 yr after a cardiac arrest associated with overwhelming sepsis. Autopsy was not performed.

Patient 3

Patient III-4 is a 24-yr-old woman who was noted at age 18 mo to have mild proteinuria and an elevated creatinine (80 μmol/L). Renal ultrasound and micturating cystogram were normal. An ophthalmologist's examination revealed bilateral optic disc coloboma. She currently has stable chronic renal impairment (creatinine 320 μmol/L), normal visual acuity, and normal hearing. A 24-h urinary protein assessment shows significant proteinuria of 4.56 g/L, and her mild hypertension (BP 140/95 mmHg) is currently managed with a calcium channel blocker. She has received genetic counseling advising a risk of 50% transmission in subsequent pregnancies and has since undergone a tubal ligation.

Patient 4

Patient IV-1 is a 3-yr-old boy who received a diagnosis antenatally of bilaterally small kidneys. Postnatal ultrasounds confirmed bilateral renal hypoplasia, and he has mild chronic renal impairment (creatinine 100 μmol/L). An ophthalmologist's examination showed bilateral optic disc coloboma. Urinalysis using "combur 10" test strips (Roche Diagnostics) at age 4 yr shows no evidence of proteinuria, and he remains normotensive. Initial hearing screening using visual reinforcement orientation audiometry is normal.

Patient 5

Patient IV-2 is a 6-mo-old twin girl who received a diagnosis antenatally of bilaterally small dysplastic kidneys, confirmed on postnatal ultrasound. She has mild renal impairment (creatinine 57 μmol/L) and an asymmetrical right optic disc coloboma (Figure 2B). Urinalysis using "combur 10" test strips (Roche Diagnostics) at age 8 mo shows no evidence of proteinuria, and she is currently being treated for mild hypertension with a calcium channel blocker (BP 104/65 mmHg). Initial

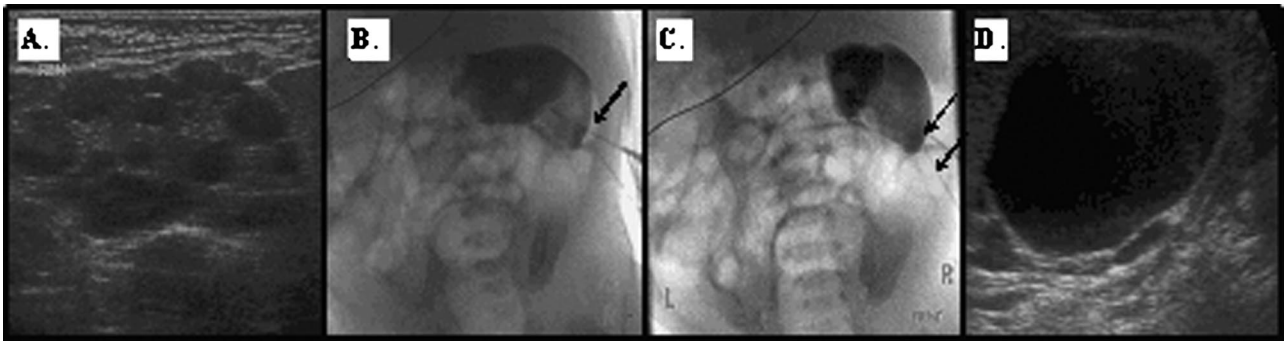

Figure 2. (A) Postnatal renal ultrasound in patient IV-3 showing multiple noncoalescing cystic structures consistent with multicystic dysplastic kidney (MCDK). (B and C) Nephrostogram performed to exclude ureteropelvic junction (UPJ) obstruction on day 2 of life shows two noncommunicating cystic structures again consistent with MCDK (small arrows indicate nephrostogram needle entering two separate cysts, confirming MCDK). (D) Renal ultrasound at 8 mo of age showing increasing size of a single cyst in the MCDK.

hearing screening using visual reinforcement orientation audiometry is normal.

Patient 6

Patient IV-3 is a 6-mo-old twin girl who received a diagnosis antenatally of a small left dysplastic kidney and right MCDK. An antegrade nephrostogram performed postnatally confirmed noncommunicating cysts, which are consistent with MCDK (Figure 3, A through C). She has moderate renal impairment (serum creatinine 99 μmol/L) and an asymmetrical right optic disc coloboma (Figure 2C). Renal ultrasound performed at 8 mo of age shows enlargement of a single cyst in the right kidney (Figure 2D). Spot urine protein shows significant proteinuria of 0.96 g/L, and she is currently treated for mild hypertension with a calcium channel blocker (BP 102/68 mmHg). Initial hearing screening using visual reinforcement orientation audiometry is normal.

Patients IV-2 and IV-3 are monozygotic with identical alleles for all nine microsatellite markers tested. Spectratype analysis showed the twins to be heterozygous at four alleles and homozygous at five alleles.

Mutation Analysis

Heterozygosity for a mutation of exon 2 of *PAX2* was identified in all affected family members by an abnormal spectratype pattern showing a normal and a shortened mutant allele. All affected individuals have identical spectratyping patterns showing two peaks; the unaffected father and normal control subjects have a single peak (Figure 4). Nucleotide sequence analysis of independent clones derived from the PCR product comprising exon 2 revealed a 10-bp deletion (nucleotide 682 to 691 of the cDNA), CAGGGTGTGC (Figure 5) within the paired box domain leading to a shift in the open reading frame. The encoded protein thus corresponds to the first 46 amino acids of *PAX2*, followed by 31 amino acids of unrelated sequence (Figure 6).

Discussion

We present six affected family members who have renal coloboma syndrome and a novel heterozygous missense mutation in the highly conserved exon 2 region of *PAX2*. A mutation encompassing this region has previously been described in a family with renal coloboma syndrome (10). Both the previously reported nucleotide deletion and the deletion presented in this report lead to a frame shift and an unrelated protein comprising 31 amino acids in length downstream of the deletion (Figure 7, A and B). Figure 7A shows the mutated protein described in this family. As can be seen in Figure 7B, the previously described larger deletion of 22 bp in the same region leads to a

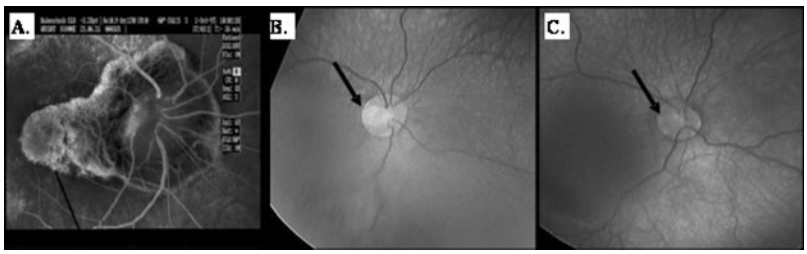

Figure 3. Phenotypic features of the eyes in patients II-3 (A), IV-2 (B), and IV-3 (C). A fluorescein angiogram of patient II-3 shows a large typical optic disc coloboma with a sharply delimited excavation occupying an enlarged optic disc. Patients IV-2 and IV-3 show the coloboma occupying the temporal aspect of optic disc with normal nasal neuroretinal rim in each patient. The colobomas were asymmetrical, with the right eye more severely affected in these two patients.

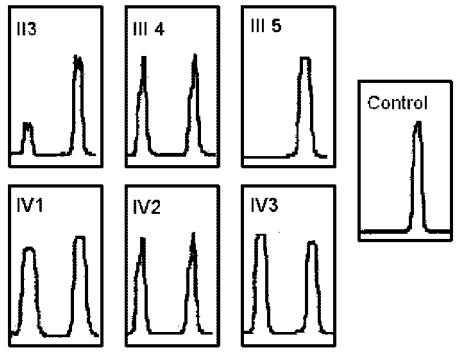

Figure 4. Spectratyping analysis of PCR products of exon 2 of *PAX2* for all tested family members and an unrelated control subject. Numbering in top right corner corresponds to the pedigree shown in Figure 1. III-5 and the control indicate the spectratype pattern showing homozygosity for exon 2. Remaining spectratype patterns show a normal and mutant allele, suggesting heterozygosity, and are from phenotypically affected family members.

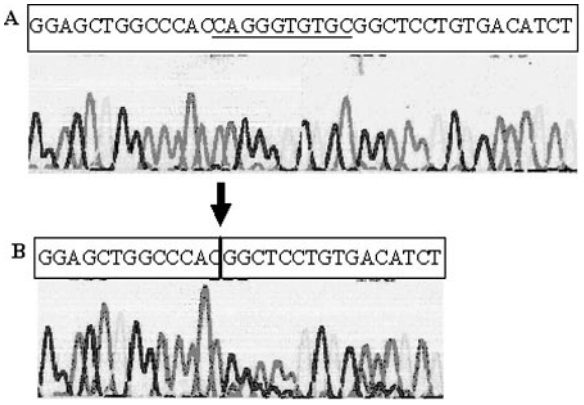

Figure 5. DNA sequence analysis in control subject and patients with renal coloboma syndrome. (A) Sequencing pattern of the control DNA showing normal *PAX2* gene. (B) Sequencing in all affected family members. DNA sequence analysis revealed a 10-bp deletion (CAGGGTGTGC) from nucleotide position 682 to 691. The nucleotides deleted introduced a stop codon (UGA) 31 amino acids downstream.

protein that is four amino acids shorter but contains the identical novel sequence generated by the frame shift.

The finding of MCDK in patient IV-3 and UPJ obstruction in patient III-3 raises several hypotheses as to the relationship of this novel *PAX2* mutation and renal obstruction. First, it strengthens the hypothesis that *PAX2* also plays a role in ureteric development, drainage, and early renal obstruction as seen in mouse models of congenital anomalies of the kidney and urinary tract (CAKUT) (30). Second, variable phenotypic expression is well documented in *PAX2* mutations in humans and mouse models (see Table 1). The variable expression of MCDK and UPJ obstruction in the *PAX2* kindred described here is consistent with the variability found in CAKUT, in which only one kidney is usually affected, whereas the other is typically spared, suggesting that there may be nongenetic factors that contribute to the development of MCDK (30,31). Alternatively, renal obstruction and MCDK may arise in these patients through co-inheritance of a second, unrelated genetic mutation. There are no other reports of digenic inheritance of either MCDK or *PAX2* mutations with other disorders. Each condition is rare, with MCDK having an incidence of 1:4300 (32) and only approximately 75 cases of *PAX2* mutations reported worldwide (1–2,4–7,9–11,20,22–24,29). Therefore, the possibility of digenic inheritance for these two conditions seems unlikely.

The possible role of *PAX2* in the development of MCDK has previously been postulated (30,33). Woolf (33) hypothesized that congenital obstruction resulting in MCDK involves the upregulation of *PAX2* from stretching of the metanephric epithelia after ureteric obstruction. This overexpression of *PAX2* may lead to an alteration in *BCL2* protein, which may stimulate renal epithelial hyperproliferation, giving rise to the kidney's multicystic appearance (33,34). This hypothesis is supported by the presence of increased *in vitro* expression of *PAX2* and *BCL2* in dysplastic epithelia (32,33). The presence of increased apoptosis in $Pax2^{1neu}$ mice heterozygous for *PAX2* mutations may also reflect the important role of *PAX2* in regulating apoptosis. In addition, increased apoptosis in homozygous congenital polycystic kidney (cpk) mice heterozygous for *PAX2* mutations has been suggested to lead to the observed slowing of the progression of renal cystic disease (35,36) in mice that carry the additional *PAX2* mutation compared with those without a *PAX2* mutation.

The presence of MCDK in patient IV-3 seems contradictory to the hypothesis that upregulation of *PAX2* results in the development of MCDK whereas downregulation slows cystic progression. Although the clinical phenotype of renal coloboma syndrome has been attributed to a decreased expression of *PAX2*, it is possible in this particular case that expression of the truncated and abnormal protein may have a dominant effect resulting in a similar process to upregulation of normal *PAX2* with obstruction. This would explain the antiapoptotic effect and cystic changes in this patient. An alternative hypothesis is that the mutant *PAX2* gene could be acting independently to cause ureteric obstruction with the development of cysts being a secondary phenomenon.

Other clinical findings in renal coloboma syndrome have raised the possibility of *PAX2* being involved in ureteric obstruction. Unilateral renal agenesis has been reported in two patients with renal coloboma syndrome. Right renal agenesis, bilateral optic nerve coloboma, and renal failure were described in a patient with an affected sibling with a less severe renal phenotype (29). This case was not diagnosed as having renal coloboma syndrome at the time of publication, although the patient's phenotype is highly suggestive of this (29). The second reported patient with renal coloboma syndrome and unilateral renal agenesis was identified to have a mutation involving a single nucleotide deletion at nucleotide position 832 of exon 3 (n. 832 del G) of *PAX2*. The same mutation in this patient's father was expressed as a less severe phenotype with renal insufficiency and reduced kidney size (1). This mutation led to truncation of the predicted paired box domain protein as

A Wild-Type mRNA and Protein

Bp 121 AUC GUG GAG CUG GCC CAC **CAG GGU GUG C**GG CCC UGU GAC AUC 162

AA 41 Ile Val Glu Leu Ala His **Gln** Gly Val Arg Pro Cys Asp Ile 54

B Predicted mRNA and Protein with mutation (n. 682-691 del 10)

Bp 121 AUC GUG GAG CUG GCC CAC GGC CCU GUG ACA UCU CCC GGC AGC 162

AA 41 Ile Val Glu Leu Ala His **Gly** *Pro Val Thr Ser Pro Gly Ser* 54

Figure 6. Protein sequence of the *PAX2* gene (AA, amino acid) and the predicted mutated sequence, with the out-of-frame sequence in italics.

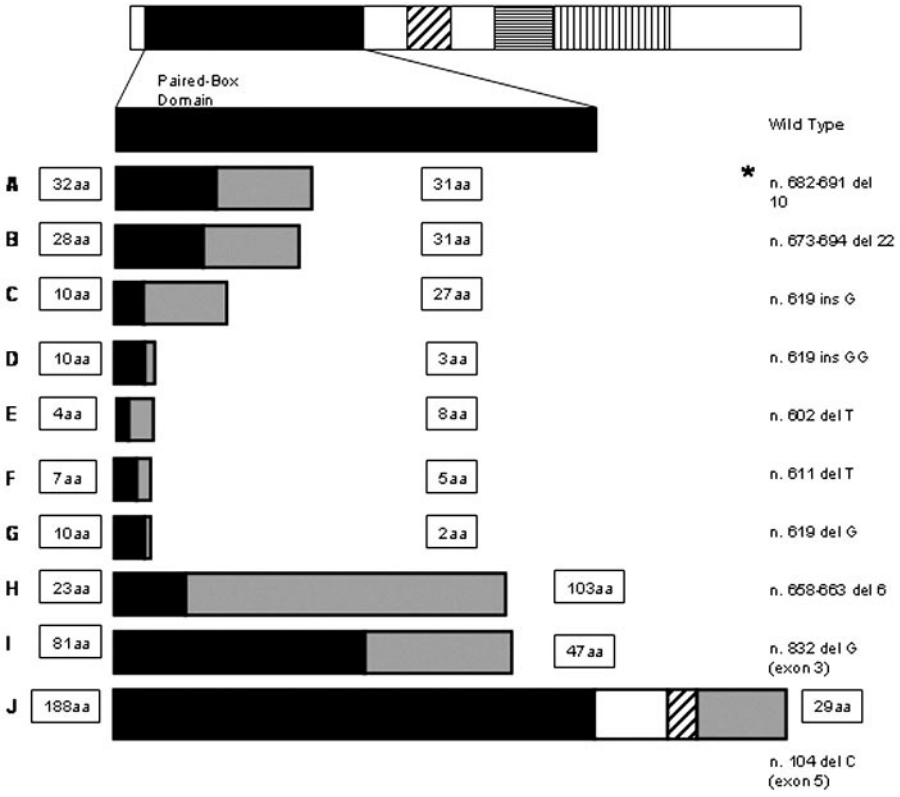

Figure 7. Schematic illustration (not to scale) of predicted *PAX2* protein truncated after mutations in exons 2, 3, and 5 of the *PAX2* gene. Top figure shows the wild-type protein with the paired box domain (■), octapeptide sequence (▨), homeodomain (▤), and transactivation domain (▥) shown. A through I show the predicted truncation of the paired box domain associated with the identified mutations in exons 2 and 3 of *PAX2*. J indicates the predicted *PAX2* protein truncated after a mutation in exon 5. Mutations are indicated on the far right; n., nucleotide; * denotes this reported mutation. Boxed numbers to the left indicate the number of amino acids (aa) corresponding to remaining wild-type protein excluding the first 15 aa before the paired box domain; boxed numbers to the right indicate the number of aa of translated mutant protein.

shown in Figure 7I. The marked clinical variability found within families and between patients with identical mutations in both structural lesions and clinical outcomes is similar to that found in patients with *WT-1* gene mutations (37). This may suggest that PAX2 is a spatially and temporally important regulator of renal development and that unilateral renal agenesis in affected patients may be caused by ureteric obstruction during early nephrogenesis, leading to involution of the affected kidney *in utero* (27,38).

Sanyanusin *et al.* (5) also report a 15-yr-old boy with a nonfunctioning right kidney and bilateral reflux. This patient was found to have a single nucleotide deletion in exon 5 (n.1104 del C). This mutation led to the paired box domain's remaining intact, potentially allowing DNA binding with truncation of the

PAX2 protein within the octapeptide sequence (Figure 7J). Potentially, mutations involving exons 3 and 5 and our mutation may allow transcription of enough amino acids to precipitate DNA binding and production of a similar mutant *PAX2* protein with aberrant *PAX2* function when compared with mutations that lead to shorter amounts of wild-type protein (Figure 7, C through H).

The observed phenotype of UPJ obstruction and MCDK in our reported patients is the first human report of a *PAX2* mutation leading to the anomalies of MCDK and UPJ encompassed in CAKUT (30,31). Several genes, including *PAX2*, have been described in mouse models as having potential roles in the ontogeny of CAKUT; these include *Kal, Eya1*, and *AGTR2* (30). The observed absence of kidneys and ureters in the *Pax2* $-/-$ mice and renal hypoplasia in the *Pax2* $+/-$ heterozygotes is in keeping with the clinical phenotype of this kindred and supports a role for *PAX2* in the ontogeny of CAKUT (30).

In conclusion, we have identified a novel mutation in exon 2 of the *PAX2* gene that causes renal coloboma syndrome and is associated with MCDK. These findings, in conjunction with the previous reports of renal agenesis in this syndrome, suggest that *PAX2* may play a role in early ureteric obstruction.

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References

1. Amiel J, Audollent S, Joly D, Dureau P, Salomon R, Tellier AL, Auge J, Bouissou F, Antignac C, Gubler MC, Eccles MR, Munnich A, Vekemans M, Lyonnet S, Attie-Bitach T: PAX2 mutations in renal-coloboma syndrome: Mutational hotspot and germ line mosaicism. *Eur J Hum Genet* 8: 820–826, 2000
2. Eccles MR, Schimmenti LA: Renal-coloboma syndrome: A multi-system developmental disorder caused by PAX2 mutations. *Clin Genet* 56: 1–9, 1999
3. 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
4. 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
5. 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
6. Devriendt K, Matthijs G, Van Damme B, Caesbroeck DV, Eccles MR, Vanrenterghem Y, Fryns J, Leys A: Missense mutation and hexanucleotide duplication in the *i* gene in two unrelated families with renal-coloboma syndrome. *Hum Genet* 103: 149–153, 1998
7. Schimmenti LA, Shim HH, Wirtschafter JD, Panzarino VA, Kashtan CE, Kirkpatrick SJ, Wargowski DS, France TD, Michel E, Dobyns WB: Homonucleotide expansion and contraction mutations of PAX2 and inclusion of Chiari 1 malformation as part of renal-coloboma syndrome. *Hum Mutat* 14: 369–376, 1999
8. Schimmenti LA, Pierpont ME, Carpenter BLM, Kashtan CE, Johnson MR, Dobyns WB: Autosomal dominant optic nerve colobomas, vesicoureteral reflux and renal anomalies. *Am J Med Genet* 59: 204–208, 1995
9. Ford B, Rupps R, Lirenman D, Van Allen MI, Farquharson D, Lyons C, Friedman JJ: Renal-coloboma syndrome: Prenatal detection and clinical spectrum in a large family. *Am J Med Genet* 99: 137–141, 2001
10. 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
11. Torres M, Gomez-Pardo E, Dressler GR, Gruss P: Pax-2 controls multiple steps of urogenital development. *Development* 121: 4057–4065, 1995
12. Keller SA, Jones JM, Boyle A, Barrow LL, Killen PD, Green DG, Kapousta NV, Hitchcock PF, Swank RT, Meisler MH: Kidney and retinal defects (Krd), a transgene-induced mutation with a deletion of mouse chromosome 19 that includes the Pax2 locus. *Genomics* 23: 309–320, 1994
13. Sanyanusin P, Norrish JH, Ward TA, Nebel A, McNoe LA, Eccles MR: Genomic structure of the human PAX2 gene. *Genomics* 35: 258–261, 1996
14. Burton Q, Cole LK, Mulheissen M, Chang W, Wu DK: The role of Pax2 in mouse inner ear development. *Dev Biol* 272: 161–175, 2004
15. Ritz-Blazer B, Estreicher A, Gauthier B, Philippe J: The paired homeodomain transcription factor Pax-2 is expressed in the endocrine pancreas and transactivates the glucagon gene promoter. *J Biol Chem* 275: 32708–32715, 2000
16. Eccles MR, He S, Legge M, Kumar R, Fox J, Zhou C, French M, Tsai RW: PAX genes in development and disease: The role of PAX2 in urogenital tract development. *Int J Dev Biol* 46: 535–544, 2002
17. Tellier AL, Amiel J, Delezoide A, Audollent S, Auge J, Esnault D, Encha-Razavi F, Munnich A, Lyonnet S, Vekemans M, Attie-Bitach T: Expression of the PAX2 gene in human embryos and exclusion in the CHARGE syndrome. *Am J Med Genet* 93: 85–88, 2000
18. Discenza WT, He S, Lee TH, Chu LL, Bolon B, Goodyer P, Eccles MR, Pelletier J: WT1 is a modifier of the Pax2 mutant phenotype: Cooperation and interaction between WT1 and Pax2. *Oncogene* 22: 8145–8155, 2003
19. McConnell MJ, Cunliffe HE, Chua LJ, Ward TA, Eccles MR: Differential regulation of the human Wilms tumour suppressor gene (WT1) promoter by two isoforms of PAX2. *Oncogene* 14: 2689–2700, 1997
20. Nishimoto K, Iijima K, Shirakawa T, Kitagawa K, Satomura K, Nakamura H, Yoshikawa N: PAX2 gene mutation in a

family with isolated renal hypoplasia. *J Am Soc Nephrol* 12: 1769–1772, 2001
21. Porteous S, Torban E, Cho NP, Cunliffe HE, Chua LJ, McNoe LA, Ward TA, Souza C, Gus P, Giugliani R, Sato T, Yun K, Favor J, Sicotte M, Goodyer PR, Eccles MR: Primary renal hypoplasia in humans and mice with PAX2 mutations: Evidence of increased apoptosis in fetal kidneys of Pax21neu +/− mutant mice. *Hum Mol Genet* 9: 1–11, 2000
22. 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
23. Chung GW, Edwards AO, Schimmenti LA, Manligas GS, Zhang YH, Ritter R: Renal-coloboma syndrome: Report of a novel PAX2 gene mutation. *Am J Ophthalmol* 132: 910–914, 2001
24. Salomon R, Tellier AL, Attie-Bitach T, Amiel J, Vekemans M, Lyonnet S, Dureau P, Niaudet P, Gubler MC, Broyer M: PAX2 mutations in oligomeganephronia. *Kidney Int* 59: 457–462, 2001
25. Eccles MR, Jacobs GH: The genetics of primary vesicoureteric reflux. *Ann Acad Med Singapore* 29: 337–345, 2000
26. Eccles MR, Bailey RR, Abbott GD, Sullivan MJ: Unravelling the genetics of vesicoureteric reflux: A common familial disorder. *Hum Mol Genet* 5: 1425–1429, 1996
27. Mesrobian HJ, Rushton HG, Bulas D: Unilateral renal agenesis may result from in utero regression of multicystic renal dysplasia. *J Urol* 150: 793–794, 1993
28. Potter EL: *Normal and Abnormal Development of the Kidney*. Chicago, Year Book Medical Publishers, 1972, pp 1–305
29. Weaver RG, Cashwell LF, Lorentz W, Whiteman D, Geisenger KR, Ball M: Optic nerve coloboma associated with renal disease. *Am J Med Genet* 29: 597–605, 1988
30. Miyazaki Y, Ichikawa I: Ontogeny of congenital anomalies of the kidney and urinary tract, CAKUT. *Pediatr Int* 45: 598–604, 2003
31. Pope JC IV, Brock JW III, Adams MC, Stephens FD, Ichikawa I: 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, 1999
32. Liebeschuetz S, Thomas R: Letter on prevalence of prenatally detected MDK. *Arch Intern Med* 77: 369, 1997
33. Woolf AS: A molecular and genetic view of human renal and urinary tract malformations. *Kidney Int* 58: 500–512, 2000
34. Winyard PJ, Risdon AR, Sams VR, Dressler GR, Woolf AS: The PAX2 transcription factor is expressed in cystic and hyperproliferative dysplastic epithelia in human kidney malformations. *J Clin Invest* 98: 451–459, 1996
35. Ostrom L, Tang M, Gruss P, Dressler GR: Reduced Pax2 gene dosage increases apoptosis and slows the progression of renal cystic disease. *Dev Biol* 219: 250–258, 2000
36. Clark P, Dziarmaga A, Eccles M, Goodyer P: Rescue of defective branching nephrogenesis in renal-coloboma syndrome by the caspase inhibitor, Z-VAD-fmk. *J Am Soc Nephrol* 15: 299–305, 2004
37. Little M, Wells C: A clinical overview of WT1 gene mutations. *Hum Mutat* 9: 209–225, 1997
38. Belk RA, Thomas DFM, Mueller RF, Godbole P, Markham AF, Weston MJ: A family study and the natural history of prenatally detected unilateral multicystic dysplastic kidney. *J Urol* 167: 666–669, 2002