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Further Delineation of Renal-Coloboma Syndrome in Patients with Extreme Variability of Phenotype and Identical *PAX2* Mutations

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Summary

Renal-coloboma syndrome is a recently described autosomal dominant syndrome of abnormal optic nerve and renal development. Two families have been reported with renal-coloboma syndrome and mutations of the *PAX2* gene. The *PAX2* gene, which encodes a DNA-binding protein, is expressed in the developing ear, CNS, eye, and urogenital tract. Ocular and/or renal abnormalities have been consistently noted in the five reports of patients with renal-coloboma syndrome, to date, but *PAX2* expression patterns suggest that auditory and CNS abnormalities may be additional features of renal-coloboma syndrome. To determine whether additional clinical features are associated with *PAX2* mutations, we have used PCR-SSCP to identify *PAX2* gene mutations in patients. We report here four patients with mutations in exon 2, one of whom has severe ocular and renal disease, microcephaly, and retardation, and another who has ocular and renal disease with high-frequency hearing loss. Unexpectedly, extreme variability in clinical presentation was observed between a mother, her son, and an unrelated patient, all of whom had the same *PAX2* mutation as previously described in two siblings with renal-coloboma syndrome. These results suggest that a sequence of seven Gs in *PAX2* exon 2 may be particularly prone to mutation.

Introduction

Renal-coloboma syndrome is a multisystem developmental disorder involving optic nerve colobomas and renal hypoplasia/insufficiency, which exhibits autosomal dominant inheritance (OMIM 120330). We have recently described two families with renal-coloboma syndrome and heterozygous *PAX2* mutations (Sanyanusin et al. 1995*a*, 1995*b*). The first mutation was a cytosine deletion from exon 5 of *PAX2* in a family with optic nerve colobomas, renal anomalies, and vesicoureteric reflux (Sanyanusin et al. 1995*b*), while the second mutation was an insertion of a guanosine within a string of seven Gs in exon 2 of *PAX2* in two siblings with optic nerve colobomas and renal anomalies (Sanyanusin et al. 1995*a*). Excluding these two families, there have been only four reports of a similar syndrome involving colobomas and renal abnormalities (Reiger 1977; Karcher 1979; Bron et al. 1988; Legros y Carrenard et al. 1992), except for multiple congenital anomaly syndromes, such as CHARGE (*c*oloboma, *h*eart anomalies, *a*tresia of the choane, *r*etarded growth and development, *g*enital anomalies, and *e*ar anomalies) association (Pagon et al. 1981) or COACH (*c*erebellar vermis hypo/aplasia, *o*ligophrenia, congenital *a*taxia, *c*oloboma, and *h*epatic fibrosis) syndrome (Verloes and Lambotte 1989). Eye defects, including colobomas, and renal abnormalities sometimes occur in association with other developmental abnormalities, either as a recognized syndrome or as isolated occurrences. Conditions such as COACH or Joubert syndrome (Saraiva and Baraitsa 1992) involve cerebellar vermis hypoplasia and ataxia, in addition to chorioretinal coloboma, retinal dystrophy, renal cysts, and a variety of other abnormalities.

The paired box gene, *PAX2*, is one of nine *PAX* genes (Stuart et al. 1993) encoding transcriptional regulators (Treisman et al. 1991) that are expressed predominantly during early embryonic development (Stuart et al. 1993; Stuart and Gruss 1995). *PAX2* is expressed in the optic and otic vesicles, the mesonephros (which later gives rise to the male and female genital tracts), kidney, spinal cord, midbrain, and hindbrain (Dressler et al. 1990; Nornes et al. 1990). Expression of *Pax2* in the developing brain of zebrafish and mice includes the rostral

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part of the cerebellum and the midbrain/hindbrain junction (Puschel et al. 1992). At present, the clinical phenotype arising from *PAX2* mutations is represented by two families in which mutations have been found (Sanyanusin et al. 1995*a*, 1995*b*). It is possible that the *PAX2* mutation syndrome includes additional features that were not present, or not fully appreciated, in the two families that have so far been studied. The fact that *PAX2* is expressed in the developing ears, genital structures, and CNS, in addition to eyes and kidneys, suggests that additional features of this syndrome may be found.

In this study, we have screened patients with variable disease associations for mutations in *PAX2* by PCR-SSCP (Orita et al. 1989). In doing so, we aimed to identify new patients with *PAX2* mutations and to further characterize the *PAX2* mutant phenotype. The additional abnormalities in the patients included high-frequency hearing loss, CNS anomalies, and/or multiple eye anomalies. One of the patients was diagnosed with a CHARGE-like syndrome because of eye, ear, and renal abnormalities, although this patient did not exhibit all the features usually found in patients with CHARGE syndrome. A second patient had a constellation of severe posterior eye defects, renal abnormalities, microcephaly, and mental retardation. The second patient's mother was also affected but had very mild eye abnormalities and late-onset renal disease. The fourth patient had ocular and renal anomalies and an abnormal electroencephalogram (EEG) pattern. The disease in the three families was of de novo occurrence, and all four patients had mutations in exon 2 of *PAX2*. The disease features of the four patients were compared to the clinical details reported previously in two families with *PAX2* mutations (Weaver et al. 1981; Sanyanusin et al. 1995*a*, 1995*b*; Schimmenti et al. 1996). Taken together, these findings expand the spectrum of known developmental defects associated with *PAX2* mutations and lead to an improved understanding of the role of *PAX2* in human development. Three of the four patients had a *PAX2* mutation in exon 2 (insG619) that was identical to a mutation described elsewhere in two siblings with renal-coloboma syndrome (Sanyanusin et al. 1995*a*), suggesting that a common mutational mechanism may be involved.

Material and Methods

PCR-SSCP and Detection of PAX2 *Mutations*

Genomic DNA from each individual was extracted from peripheral blood with a DNA extraction kit (Promega) or by salt extraction. Fragments spanning exons 1–10 and exon 12 of *PAX2* were amplified from genomic DNA by use of PCR primers in the introns flanking the exons (see table 1). The primer sequences used for SSCP were based on the intron sequences flanking each exon (Sanyanusin et al. 1996). The PCR products were labeled by incorporation of ^{32}P-dCTP in reactions containing 100 ng DNA, 62.5 μM dNTPs, 1 μCi [α^{32}P] dCTP (3,000 Ci/mmol), 20 pmol of each primer, reaction buffer (50 mM KCl, 10 mM Tris pH 8.3, 1.0–3.0 mM $MgCl_2$), and Hot Tub (Amersham), Pwo (Boehringer Manheim), or *Taq* (Perkin Elmer) DNA polymerase. Amplification conditions are given in table 1. Following 30 cycles of amplification, the PCR products were electrophoresed in nondenaturing 6% or 12% polyacrylamide gels (Sambrook et al. 1989) with 1 × Tris-borate EDTA, pH 8.0 buffer to reveal single-strand conformational variants (Orita et al. 1989). Several conditions of polyacrylamide gel electrophoresis were used, including gels with or without 10% glycerol, and electrophoresis at 25°C or at 4°C. Gels with nonlabeled PCR products were visualized by silver stain with modification.

Subcloning and DNA Sequencing

PCR products were directly sequenced using α^{35}S-dATP or α^{32}P-dCTP and a cycle sequencing kit (GIBCO-BRL), or, alternatively, PCR products were directly sequenced, after purification using a QIAquick gel-extraction kit (Qiagen), on a model 377 automated sequencer (ABI). For subcloning of exon 2 PCR products, mutant and normal alleles of *PAX2* were amplified using Pwo DNA polymerase (Boehringer Manheim) and subcloned into *Eco*RV-digested pBluescript II (Stratagene) or pCRII (Invitrogen). Positive clones were identified by blue/white selection, and plasmid DNA was isolated using Wizard mini-preps kits (Promega) or a QIAprep Spin Plasmid kit (Qiagen). Sequencing reactions were carried out on plasmid DNA by use of ^{35}S-dATP and a double-stranded sequencing kit (USB) or on a model 377 ABI automated sequencer.

Allele-Specific Oligonucleotide Hybridization

PAX2 exon 2 was amplified from patient 2646, her parents, and three control patients. Allele-specific oligonucleotide analysis was performed by the method described in Sylvester-Jackson et al. (1993). Denatured PCR products were dotted by vacuum using a Bio-Dot Microfiltration Apparatus (Bio-Rad) onto BIODYNE B membrane (Gibco-BRL) and cross-linked with UV light. Allele-specific oligonucleotides were synthesized (ABI) with the following sequences: normal allele: ACCAGC-TCGGGGGGGTGTTT and mutant allele: ACCAGC-TCGGGGGGGGTGT (T_m = 66°C). Oligonucleotides were end-labeled using T4 polynucleotide kinase (Amersham) and γ-^{32}P-ATP (6,000 Ci/mmol) (Amersham) (Sambrook et al. 1989). Prehybridization and hybridization were as described by Sylvester-Jackson et al. (1993).

Clinical Evaluations

Individuals were evaluated by history, physical examination, routine urine and blood studies, ophthalmologic

Table 1

***PAX2* PCR-SSCP Primer Sequences and Reaction Conditions**

Exon[a]	Sequence	Magnesium Concentration	T_m (°C)	Product Size (bp)
1F	5′ GTT CAC TCA TCC TCC CTC CCC CAC C 3′			
1R	5′ GGA GCC GGG CGC GGG TAC TC 3′	1.5	60	179
2F	5′ CTG TGT GTG GGG TGT TGT GTT 3′			
2R	5′ AAG GCG TCT CTC CCG GGA CAG CTG C 3′	2.0	58	246
3F	5′ TGA CCG GCT TTC CCG GCG CA 3′			
3R	5′ GAG GAA GCT GGA GTC CAG CC 3′	1.25	60	262
4F	5′ TGG AGC TGC GTT TCC TGC CTT 3′			
4R	5′ AAT TGG CCG GAA TAG CAG TGG 3′	3.0	61	210
4S	5′ CGG AAT AGG AGT GGC ATT TGA 3′			
4A	5′ CTC TAG GTG GGA TCT GGT TT 3′	1.25	58	181
5F	5′ TGA TGC CAT TTC CTC CTT CC 3′			
5R	5′ GCC ACA CCT CTT CCC TCC T 3′	1.0	60	175
6F	5′ CAG TGT TTG TCT GTC TCT TAT TTG CT 3′			
6R	5′ ATG TTC CCT CTG GCC CTC A 3′	1.0	52	121
7F	5′ CGC CCC GAG TGT CCA TGT GTT 3′			
7R	5′ TAC TTC TGC AAG CAG AAA GCT CCC T 3′	1.5	58	234
8F	5′ CCT TTC TCT GTG CGT GCA TCA ATA GA 3′			
8R	5′ GGC ACC CTC CAC TGA ACG CAG 3′	1.5	58	227
9F	5′ CCC TTC CCC TTT GTG TTT TT 3′			
9R	5′ AGG CAG CTG CAG CAT TGT C 3′	1.5	60	151
10F	5′ CCCCTCCCTGCAAACCAC 3′			
10R	5′ CGCTGTGAGGGCCATGAC 3′	2.0	57	150
12S	5′ TCT GAC CCA GCC CAT TCT TCT 3′			
12A	5′ ATG TGG AGG CCG AAG CTG T 3′	1.25	58	163
12F	5′ CCTCTGACCCAGCCCATTC 3′			
12R	5′ GTCATAGGCAGCGGCACG 3′	1.5	60	105

[a] F = forward primer; R = reverse primer; S = alternative forward (sense) primer; A = alternative reverse (antisense) primer.

examination, renal ultrasound, and intravenous pyelogram for kidney function. Renal biopsies were taken at the University of Missouri, Columbia.

Results

Clinical Reports of the Patients with PAX2 *Mutations*

Patient 579 is an 11-year-old male born after an uncomplicated pregnancy. At birth, his weight was 3,040 g and length, 42.5 cm. At age 3 mo, his weight gain was poor, and he had polyuria, with a urine volume of 500–600 ml/d, severe proteinuria (101.8 mg/dl), and hypertension (120/74 mmHg). Serum creatinine was 5.24 mg/dl, and blood urea nitrogen was 94.6 mg/dl. At age 2 years, progressive end-stage renal failure developed, and he required peritoneal dialysis. A renal biopsy was not done. The patient is currently on peritoneal dialysis therapy. Cardiac echocardiography was normal, but renal ultrasound showed bilateral renal hypoplasia (right, 26 mm; left, 30 mm). Bilateral retinal and optic nerve colobomas were detected at 3 years of age. His visual acuity at age 11 years was 20/120 in the left eye and 20/500 in the right eye. By ultrasound, his heart was normal, but EEG showed an abnormal spike and wave complex during sleep. His IQ at 9 years was 91, which was within the normal range. At age 9 years, he had his first clonic seizure. Magnetic resonance imaging (MRI) and computed tomography (CT) examination at 5 and 7 years were normal. He had no specific developmental or behavioral abnormalities. External ear anomalies or hearing loss were not present. His karyotype was 46, XY. There was no family history of note, and his two siblings were unaffected.

Patient 656, the 48-year-old mother of patient 657, had hypertension and proteinuria during two pregnancies (ages 19 and 22 years). End-stage renal disease and bilateral renal hypoplasia were diagnosed at age 24 years, and she is currently maintained on hemodialysis waiting for a second renal transplant after failure of her first transplant. Bilateral opacities of the anterior and posterior lens capsules were noted, later requiring surgical treatment. The fundi of both eyes showed a hypoplastic optic disc. Her visual acuity is 20/30 in both eyes. She has normal intelligence, normal hearing, and no history of seizures, but no brain imaging has been performed. Her physical exam was significant for soft skin, as was noted in her son (patient 657).

Patient 657 is a 25-year-old male who was moderately

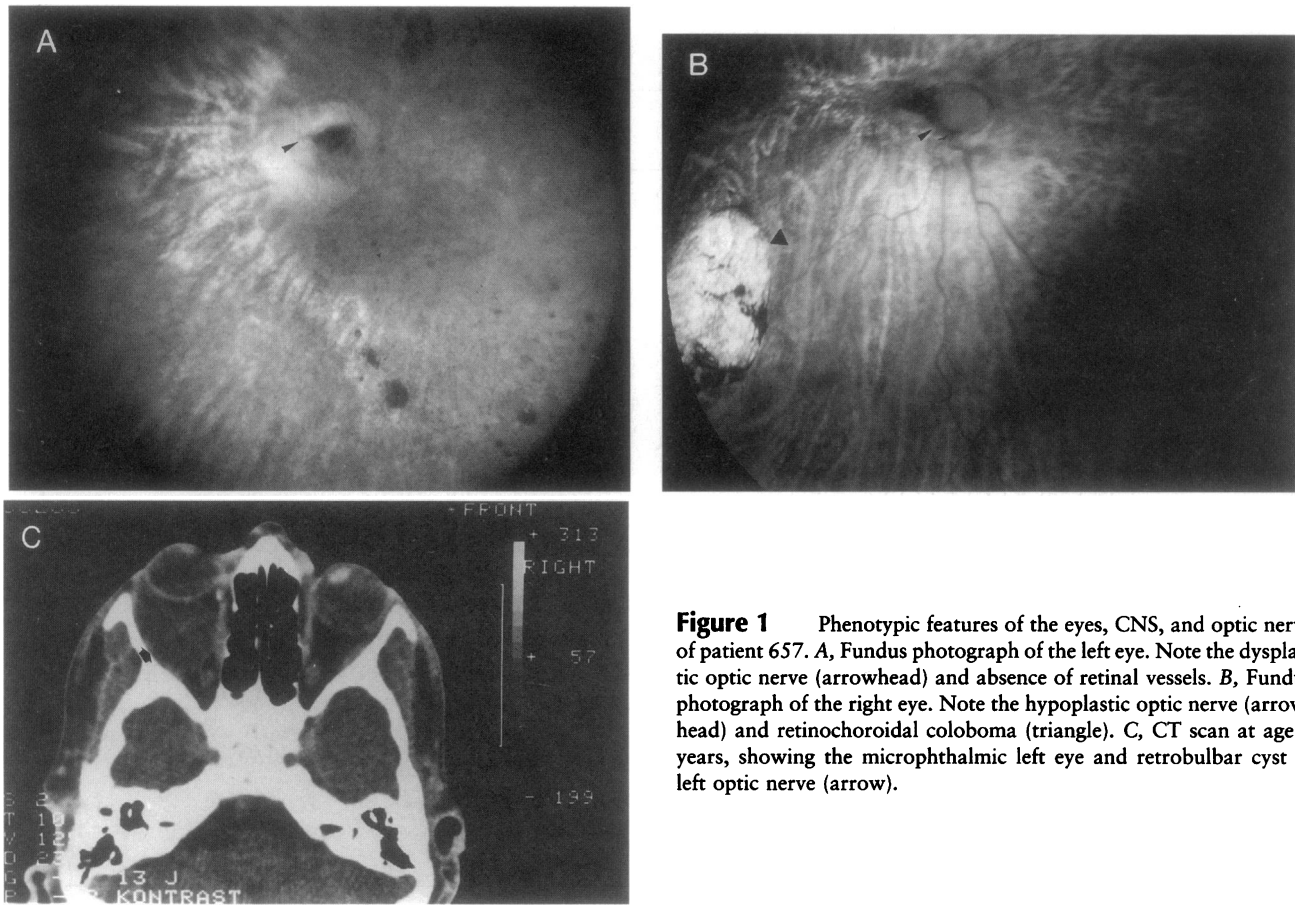

Figure 1 Phenotypic features of the eyes, CNS, and optic nerve of patient 657. *A*, Fundus photograph of the left eye. Note the dysplastic optic nerve (arrowhead) and absence of retinal vessels. *B*, Fundus photograph of the right eye. Note the hypoplastic optic nerve (arrowhead) and retinochoroidal coloboma (triangle). *C*, CT scan at age 7 years, showing the microphthalmic left eye and retrobulbar cyst in left optic nerve (arrow).

mentally retarded and microcephalic. At age 3 mo, he presented with esotropia, exophthalmos, and no direct pupillary response to light stimulation of the left eye. The anterior segments of both eyes were normal. The fundi of both eyes were lightly pigmented. In the left eye, no optic nerve head and no retinal vessels were present. In the area where the disc is normally present, a gray structure with an overlying pit was evident. In the right eye, milder changes were observed, including a hypoplastic optic disc with a pit surrounded by pigment, diffuse atrophic changes of the retina, and retinochroroidal colobomas in the inferior fundus. Electroretinography was subnormal in the right eye, and responses were electronegative in the left eye. The fundus abnormalities remained nearly unchanged during follow-up (figs. 1*A* and *B*). At age 7 years, ultrasound and CT scans showed a retrobulbar cyst behind the left microphthalmic eye (fig. 1*C*). The left eye had no light perception: mild posterior capsular opacities and incipient band keratopathy were noted and remained unchanged during follow-up. Visual acuity of the right eye was 20/300. At age 4 mo, renal insufficiency was noted with hypoplastic kidneys, which deteriorated, and a kidney transplant was required by age 14 years. MRI of the brain at age 25 years showed no additional anomalies. His karyotype was 46, XY, and hearing was normal. He had soft skin but no dysmorphic features.

Patient 2646 is a 20-year-old female who developed proteinuria at age 3 years and progressed to end-stage renal disease. She was the product of a 34-wk gestation secondary to a partial placental abruption. Her birth weight was 1,820 g. She spent 6 wk in the neonatal intensive care unit, and her first year was punctuated by numerous upper respiratory infections. Her renal disease progressed. At age 8 years, she had a normal intraveous pyelogram. At age 10 years, a 24-h urine sample contained 730 mg total protein. She underwent renal biopsy, which demonstrated focal segmental glomerulosclerosis (fig. 2), and renal ultrasound showed small kidneys. By age 18 years, she received a renal transplant from her brother. She rejected this transplant and is currently maintained on hemodialysis and recently received a cadaveric transplant. Her blood pressure was moderately elevated at 148/93 mmHg. At age 6 years, she was treated for amblyopia with patching, at which time bilateral optic nerve colobomas were discovered (fig. 2). At present, she has relatively good vision and does not require corrective lenses. Other medical prob-

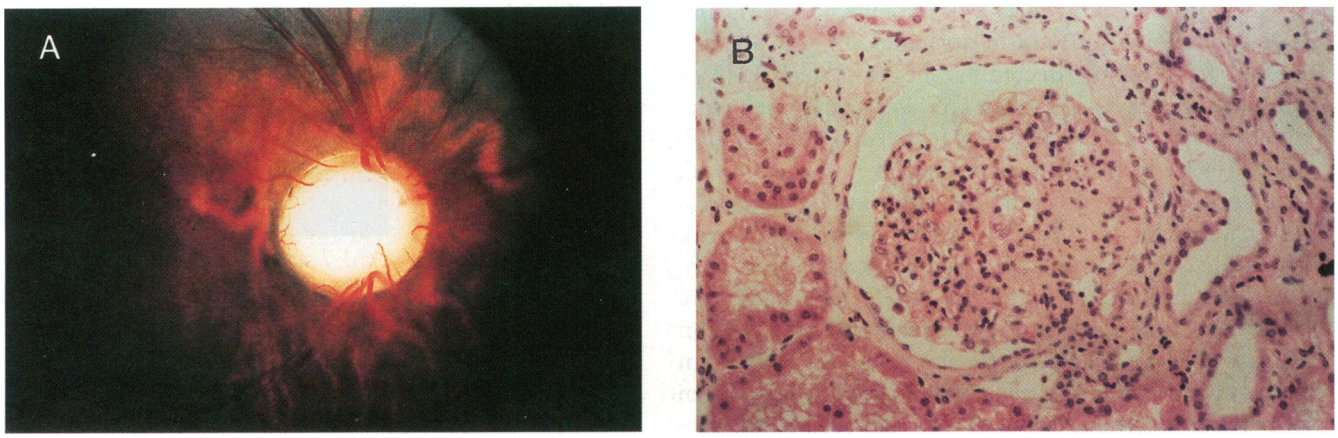

Figure 2 *A*, photograph of the left fundus of patient 2646 showing the optic nerve coloboma. *B*, Light microscopy of renal biopsy specimen from patient 2646. The glomerulus exhibits mesangial fibrosis and glomerulosclerosis. H & E stain. ×200.

lems include repair of bilateral inguinal hernea at age 6 years, asthma, and chronic sinusitis. Developmentally, she graduated from high school and has attended a community college. However, she reports learning difficulties in reading and math, requiring extra help. She has a history of depression but no seizures. Her physical examination is significant for normal height and weight. She has mild dysmorphism consisting of superior ear helices that are hypoplastic and overfolded. Her palpebral fissures are upslanting, and her nose has a bulbous tip. Her gingiva were hyperplastic. Her joints were hyperextensible. Audiological evaluation demonstrated right-sided hearing loss in the high-frequency range (6,000–8,000 Hz). Karyotype was 46, XX.

Mutational Analyses

Genomic DNA was isolated from each individual and analyzed for *PAX2* mutations. All 12 exons of *PAX2*, except exon 11, were amplified by PCR with oligonucleotide primers with optimized protocols (table 1). The products were analyzed by SSCP analysis and, in some cases, by direct cycle sequencing. Abnormal SSCP pat-

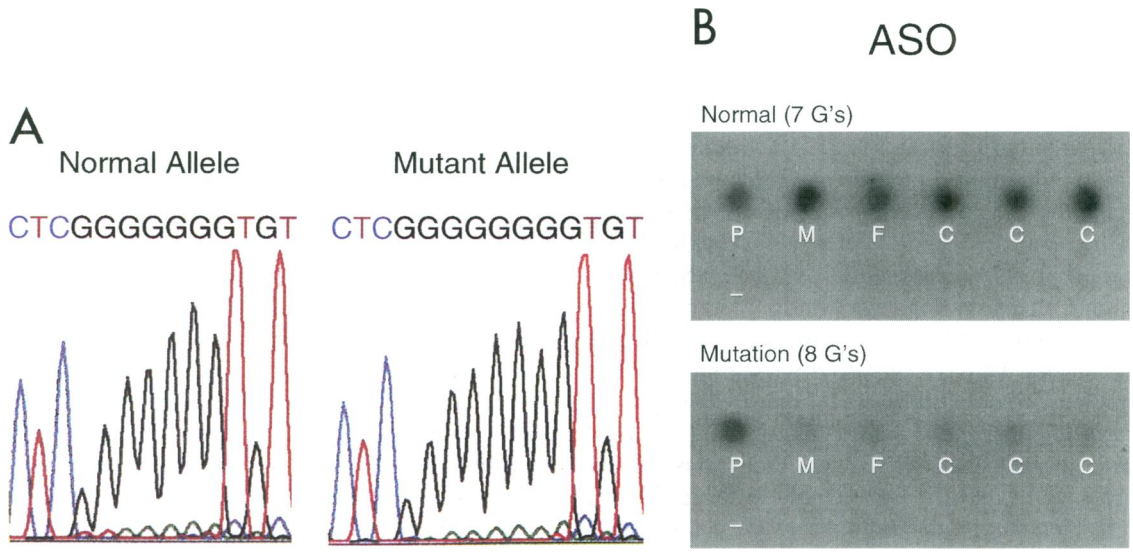

Figure 3 Detection of identical *PAX2* mutations in patients 656, 657, and 2646. *A*, Representative sequence analysis of cloned mutant alleles of *PAX2* exon 2 from patients 656, 657, and 2646. Shown is the mutant *PAX2* sequence of patient 2646, compared with a normal allele from the same patient. The mutation is an insertion of a guanosine in the paired box domain. *B*, Allele-specific oligonucleotide (ASO) hybridization to DNA from patient 2646. Oligonucleotides were designed to detect *PAX2* exon 2 alleles with seven Gs (*row i*) or eight Gs (*row ii*), respectively. All the DNA samples hybridized with the 7G oligo (see *row i*), whereas only patient 2646, who was heterozygous for the mutation, hybridized with the 8G oligo (see *row ii*).

terns were observed in exon 2 of patients 656, 657, and 2646 (not shown) but not in normal control DNA, nor in DNA from the unaffected parents of patient 2646 or the unaffected sister of patient 657. Direct cycle sequencing of the exon 2 PCR products from each affected individual revealed an identical mutation in patients 656, 657, and 2646 involving an insertion in a string of Gs and a different mutation in patient 579 (not shown). The exon 2 PCR products from each patient were subcloned, and multiple clones from each individual were sequenced. Independently derived mutant alleles from patients 656, 657, and 2646 were found to contain an insertion of a gunaosine nucleotide in the string of seven Gs between positions 613 and 619 (fig. 3*A*). Allele-specific oligonucleotides were hybridized to DNA from patient 2646, her parents, and control individuals, showing that patient 2646 had both a mutant allele containing eight Gs and a normal allele containing seven Gs (fig. 3*B*). An additional band was observed in the PCR product of patient 579 (fig. 4*A*). Mutant alleles from patient 579 were found to contain a deletion of 22 nt between positions 674 and 695 of *PAX2* exon 2, inclusive (fig. 4*B*). Normal alleles were also present in cloned DNA from each of the affected patients. The mutations identified in these patients are summarized in figure 5.

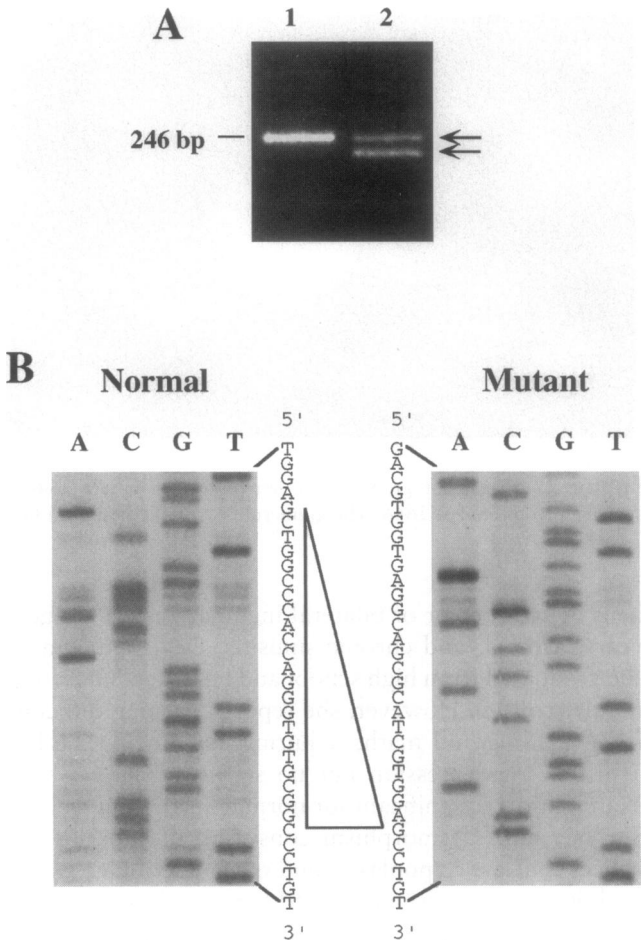

Figure 4 Detection of a 22-bp deletion from exon 2 of *PAX2* in patient 579. *A*, Ethidium bromide–stained gel of the PCR product from patient 579, generated using exon 2 primers (table 1). Lane 1, Unaffected control DNA. Lane 2, Patient 579 DNA. Note the smaller band in addition to the normal-sized band (arrows). *B*, Sequence analysis of a cloned mutant and normal allele from patient 579. Shown is the mutant sequence with a 22-bp deletion, and a normal sequence from patient 579.

Discussion

This report describes four individuals with *PAX2* mutations who had highly variable clinical features. From these findings, we have broadened the spectrum of abnormalities observed in patients with *PAX2* mutations. We compared the phenotypes of the four patients with *PAX2* mutations in this study with six patients previously characterized as having *PAX2* mutations (Sanyanusin et al. 1995*a*, 1995*b*) (table 2). The additional features of the *PAX2* mutant syndrome that we observed in patients with *PAX2* mutations include multiple posterior eye abnormalities, high-frequency hearing loss, soft skin and joint abnormalities, and possibly CNS anomalies. Although these findings are almost certainly influenced by ascertainment bias, it is interesting to compare the abnormalities we have observed with abnormalities present in *Pax2* knockout mice (Torres et al. 1995, 1996). *Pax2* homozygous mutant mice lacked kidneys, ureters, and genital tracts, whereas heterozygous mutant mice frequently developed hypoplastic kidneys (Torres et al. 1995). Heterozygous *Pax2* mutant mice also exhibited exencephaly relatively frequently, dependent on the background strain, but only some had optic nerve colobomas (Torres et al. 1996). In contrast, homozygous *Pax2* mutant mice frequently had abnormalities of the optic stalk, failure of the optic fissure to close, agenesis of the cochlea and spiral ganglion in the inner ear, and exencephaly resulting from failure of neural tube closure at the midbrain region (Torres et al. 1996).

The 4 patients with *PAX2* mutations analyzed here were among 40 patients from 29 families with abnormalities including ocular and renal defects. These patients were chosen for *PAX2* mutational analysis because either the patient's syndrome resembled renal-coloboma syndrome, the oculorenal abnormality was undiagnosed, or the genetic cause of the syndrome was unknown. The frequency with which we detected *PAX2* mutations was ~10%, although it should be noted that we did not restrict our analysis to one particular phenotype. In at least two families the syndrome closely resembled renal-coloboma syndrome, but we were unable to identify a *PAX2* mutation. It is possible that in these families we missed the *PAX2*

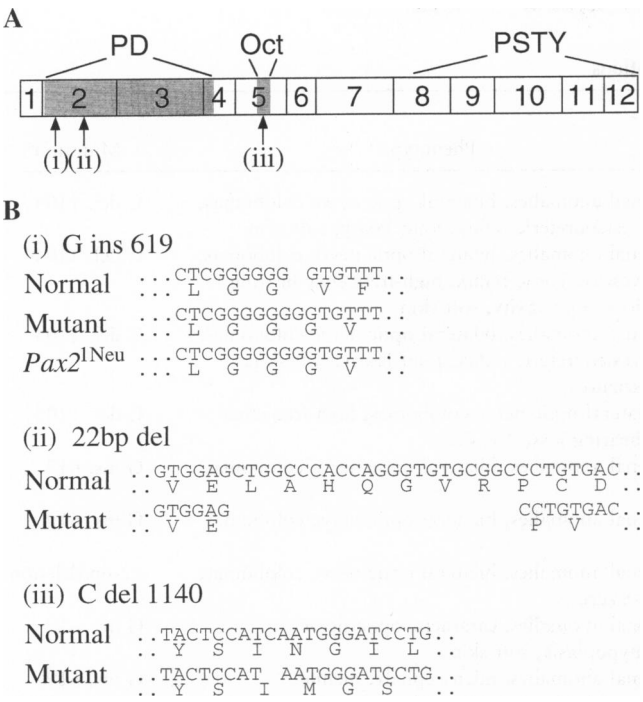

Figure 5 *A*, Schematic representation of the *PAX2* coding region (exons 1–12). Positions of the paired (PD), octapeptide (Oct), and PSTY domains are shown. The positions of the three mutations reported, to date (Sanyanusin et al. 1995*a*, 1995*b*; present study), are indicated by arrows. The shaded portions show the paired domain (PD) in exons 2, 3, and 4 and the octapeptide (Oct) sequence in exon 5, respectively. *B*, Partial nucleic acid and corresponding amino acid sequences for normal and mutant alleles, corresponding to the three characterized *PAX2* mutations. Also shown is *Pax2*^{1Neu}, a murine *Pax2* mutation, which is identical to a *PAX2* mutation in humans (Sanyanusin et al. 1995*a*; present study). Nucleotide positions refer to the *PAX2* cDNA sequence (Eccles et al. 1992).

mutation, because neither exon 11 nor the *PAX2* promoter was analyzed.

Overall, optic nerve and renal abnormalities occurred in almost all of the patients in whom *PAX2* mutations have been so far described (table 2). The optic nerve abnormalities varied widely among the 10 patients, with patient 657 having the most severe eye abnormalities, but, paradoxically, his mother (patient 656), who had the same *PAX2* mutation, exhibited very mild eye abnormalities. We have no explanation for the wide variation in phenotypic severity of the eye disease from one generation to the next, except that differences in the genetic background of mother and son may in part account for it. Renal abnormalities in the 10 patients consistently led to end-stage renal disease, except in patient 4 (table 2), whose only evidence of renal anomalies was renal hypoplasia and hypertension. At a lower frequency, abnormalities of the skin, joints, and ears, including sensorineural hearing loss, were present. High-frequency hearing loss occurred in three patients (patients 2, 4, and 2646; table 2) and may result from a developmental disturbance of the membranous labyrinth, a structure derived from the otic placode (Noden and van de Water 1992). It is possible that the renal pathology, colobomas, and hearing loss in some patients with *PAX2* mutations may be diagnosed as a CHARGE-like syndrome, as was the case with patient 2646, even though this patient did not have other findings such as congenital heart disease. Six of the 10 patients (patients 1–4, 656, and 657, table 2) had soft skin, and 4 of 10 patients (patients 1–3 and 2646, table 2) had joint laxity, indicating either that they had specific anomalies in skin and joint tone or of extracellular matrix proteins. Since *PAX2* is not known to be expressed in these tissues (Dressler et al. 1990; Nornes et al. 1990), the reasons for these effects are unclear.

CNS abnormalities were found in two patients with *PAX2* mutations (patients 579 and 657, table 2). Although the etiology of these findings is unclear, patients 579 and 3 (table 2) were both reported to have seizure disorders, and patient 657 in this study was mentally retarded. No other apparent explanation could be found for the mental retardation in patient 657. However, while CNS abnormalities, including exencephaly, were observed in *Pax2* mutant mice (Torres et al. 1996), further evaluation of patients with *PAX2* mutations is necessary to determine whether mental retardation, or other CNS abnormalities, is a true association with *PAX2* mutations in humans. In addition, patient 657 had a retrobulbar cyst, which is a very uncommon finding, although retrobulbar cysts are sometimes seen in Joubert syndrome (van Dorp et al. 1991). In patient 657, the retrobulbar cyst was thought to represent a neurogenic anomaly of the optic nerve, perhaps because of abnormal closure of the embryonic optic fissure. The optic fissure extends into the optic stalk, which is a region of *Pax2* expression in mice. In addition to closure of the optic fissure, the expression of *Pax2* appears to be required in the optic stalk for the establishment of axonal pathways and in determining the trajectories of axonal fibers growing into the chiasma region (Torres et al. 1996).

Two *PAX* genes, *PAX3* and *PAX6*, have previously been implicated in Waardenburg syndrome and anterior chamber anomalies/aniridia, respectively (Ton et al. 1991; Baldwin et al. 1992; Glaser et al. 1992; Jordan et al. 1992; Tassabehji et al. 1992). The *Pax3* gene is coexpressed with *Pax2* in the developing ear of 9-d post coitum (p.c.) mice (Nornes et al. 1990; Goulding et al. 1991). Homozygous *Pax3* mutant (splotch) mice die in utero and have defects of the membranous labyrinth, the receptors of the inner ear, white spotting of the abdomen and limbs, spina bifida, cardiac abnormalities, and anomalies of the peripheral nervous system (Epstein et al. 1991). Many of these abnormalities are consistent

Table 2

Comparison of Phenotypic Abnormalities in 10 Patients with *PAX2* Mutations

Patient	Age (years)	Pedigree	Reference	Phenotype	Mutation[a]
1	14	A	Sanyanusin et al. (1995*b*) (patient 1)	Renal anomalies, bilateral optic-nerve colobomas, vesicoureteric reflux, joint laxity, soft skin	C del. 1104
2	10	A	Sanyanusin et al. (1995*b*) (patient 2)	Renal anomalies, bilateral optic nerve colobomas, vesicoureteric reflux, high-frequency hearing loss, joint laxity, soft skin	C del. 1104
3	6	A	Sanyanusin et al. (1995*b*) (patient 3)	Renal anomalies, bilateral optic-nerve colobomas, vesicoureteric reflux, joint laxity, soft skin, seizures	C del. 1104
4	35	A	Sanyanusin et al. (1995*b*) (patient 4)	Bilateral optic-nerve colobomas, high-frequency hearing loss, soft skin	C del. 1104
5	5	B	Sanyanusin et al. (1995*a*) (patient 1)	Renal anomalies, bilateral optic-nerve colobomas	G ins. 619
6	12.5	B	Sanyanusin et al. (1995*a*) (patient 2)	Renal anomalies, bilateral optic-nerve colobomas	G ins. 619
579	11	...	Present study	Renal anomalies, bilateral optic-nerve colobomas, seizures	22-bp deletion
656	48	C	Present study	Renal anomalies, cataracts, optic-nerve hypoplasia, soft skin	G ins. 619
657	25	C	Present study	Renal anomalies, microcephaly, mental retardation, microphthalmos, retrobulbar cyst, optic-nerve aplasia, optic pits, retinochoroidal colobomas, capsular opacities, soft skin	G ins. 619
2646	20	...	Present study	Renal anomalies, bilateral optic-nerve colobomas, high-frequency hearing loss, external ear anomalies, joint laxity, asthma	G ins. 619

[a] C del. 1104 = cytosine deletion at position 1104; G ins. 619 = guanosine insertion at position 619.

with a loss of migration-competent neural crest precursor cells (Moase and Trasler 1990). In humans, heterozygous *PAX3* mutations are associated with Waardenburg syndrome, approximately one third of whom have sensorineural hearing loss at all sound frequencies (Hoth et al. 1993). In our study, *PAX2* mutations were associated with high-frequency hearing loss in 3/10 patients, which is in agreement with the identification of inner-ear abnormalities in *Pax2* knockout mice (Torres et al. 1996). Although heterozygous *Pax2* mutant mice did not show obvious structural ear abnormalities, in homozygous mutant mice the cochlea and cochlear ganglion failed to develop (Torres et al. 1996). It appears that expression of both *PAX2* and *PAX3* are necessary for ear development, and mutation in either gene may result in sensorineural hearing loss in humans. It is also of interest that neural crest cells, which may be specified by *PAX* gene expression, are required for the positioning of the tympanum and the subsequent morphogenesis of the outer ear (Noden 1983). In this respect, both patients 1 and 2646 (table 1) had a characteristic ear shape, and patient 2646 had overfolded superior helices.

A different *Pax* gene, *Pax6*, is coexpressed with *Pax2* in the developing eye of 8.5-d p.c. mice (Nornes et al. 1990; Walther and Gruss 1991). In both humans and mice, *PAX6* mutations have been shown to affect the development of a wide range of eye structures (Hanson and van Heyningen 1995; Azuma et al. 1996). Moreover, in *Drosophila*, the *Pax6* homologous gene, *eyeless*, has been shown to be essential for eye development and capable of inducing ectopic eyes (Halder et al. 1995). Abnormalities of the anterior portion of the eye predominate in patients with aniridia and Peters anomaly, which have been shown to be caused by *PAX6* mutations (Hanson et al. 1994). Other eye anomalies have also been observed in aniridia patients, including retinal, optic nerve, foveal, lens, and corneal abnormalities (Nelson et al. 1984). In contrast, the results described here show that *PAX2* mutations are frequently associated with abnormalities in the posterior segment of the eye, including the optic nerve and retina. Our findings are consistent with an important role for *PAX2* in posterior eye development. By comparison, in homozygous *Pax2* mutant mice, the absence of *Pax2* led to poor optic fissure closure, failure of optic nerve glial cells to differentiate, and absence of optic chiasma (Torres et al. 1996). Further studies of the role of *PAX2* and *PAX6* in eye development are required to determine the relative importance of these two genes in eye organogenesis.

The mutations described here were of two types: a

G insertion in a string of seven Gs between nucleotide positions 613 and 619 of *PAX2* exon 2 in patients 656, 657, and 2646, and a 22-bp deletion from nucleotide positions 674–695 of exon 2 in patient 579 (summarized in fig. 5). Both mutations are predicted to cause haploinsufficiency of *PAX2* as a result of frameshift and truncation of the predicted protein within the N-terminal portion of the paired box DNA-binding domain. The G-insertion mutation has been observed previously in two siblings (Sanyanusin et al. 1995*a*) who were unrelated to these patients. Therefore in 5 of 10 patients who have markedly variable phenotypes, we have now identified the same G-nucleotide insertion. The three patients reported here, who have the same G-insertion mutation, live in geographically different locations (the United States and Belgium). Therefore, it seems unlikely that there has been a founder mutation, and indeed for patient 2646 both parents are genotypically normal. This is a highly unexpected and unusual finding, both in terms of a recurrent mutation in the same oligo-G string and from a clinical point of view. This demonstrates the extreme variability in expression of the *PAX2* mutant phenotype, even in patients with the same mutation, and that exon 2, and, in particular, the sequence of seven Gs in exon 2 may be a "hot spot" for *PAX2* mutations. It is interesting that an identical G-insertion mutation to the one described here has arisen in mice, resulting in optic nerve, kidney, CNS, and ear anomalies (*Pax2*^{1Neu}; Genbank accession Y07617; Favor et al. 1996). The resulting phenotype in *Pax2*^{1Neu} mice is very similar to the abnormalities that have been described in *Pax2* knockout mice (Torres et al. 1995, 1996). It is possible that the G-insertion mutations have arisen de novo from polymerase slippage, since single-nucleotide DNA stretches show a tendency toward polymorphism (Fry and Loeb 1992; Dawson et al. 1993).

The approach that we have taken in this study has been to identify patients with a range of clinical features that are consistent with a predicted *PAX2* mutant phenotype and then to identify the patients who had a mutant *PAX2* genotype. This approach differs from the usual, where gene mutations are generally characterized in patients with a clinically well-defined disease. At the outset of our study, we did not know the boundaries of the *PAX2* mutation syndrome. The results of this study suggest that optic nerve and retinal colobomas, combined with renal hypogenesis and renal failure, are consistent features of the syndrome and that some patients may also have sensorineural hearing loss. It is quite possible that there is a larger population of patients with isolated colobomas, renal hypogenesis, end-stage renal disease, and/or sensorineural hearing loss or, alternatively, CHARGE- or COACH-like syndromes, who have *PAX2* mutations, although in this study we have not analyzed patients with isolated ocular, auditory, or renal abnormalities. It is clear that studies of patients for *PAX2* mutations should be extended to include such individuals, including those who have additional unusual findings. Identification of additional patients with *PAX2* mutations will help to further characterize the clinical phenotype of the mutant human *PAX2* gene.

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