

The prevalence of PAX2 mutations in patients with isolated colobomas or colobomas associated with urogenital anomalies

H E Cunliffe, L A McNoe, T A Ward, K Devriendt, H G Brunner, M R Eccles

Abstract

The PAX2 gene is mutated in patients with ocular colobomas, vesicoureteral reflux (VUR), and kidney anomalies (renal-coloboma syndrome, OMIM 120330). The three abnormalities which make up this syndrome also occur in isolation, but the causal genes are not known. PAX2 encodes a transcription factor of the paired box class of DNA binding proteins, important for the development of the urogenital tract, optic nerve and adjacent retina, inner ear, and CNS. In this paper we have investigated the prevalence of PAX2 mutations in patients with ocular colobomas, microphthalmos, or retinal anomalies, either in isolation or with associated urogenital anomalies. Using PCR-SSCP, most or all exons of PAX2 were examined in blood DNA from 99 patients who have either ocular anomalies alone or a combination of ocular and urogenital conditions. PAX2 mutations were not detected in patients with ocular colobomas, either in isolation or with associated abnormalities, except in one patient with typical renal-coloboma syndrome. We conclude that PAX2 mutations are unlikely to be common in patients with ocular colobomas in isolation or in patients with ocular colobomas and associated anomalies, except for patients with typical renal-coloboma syndrome where PAX2 is known to be the aetiological cause.

(J Med Genet 1998;35:806-812)

Keywords: PAX2; renal-coloboma syndrome; coloboma; vesicoureteric reflux

Ocular colobomas (gaps or fissures in the structures of the eye) are a relatively common malformation which can occur either sporadically or as an autosomal dominantly inherited malformation involving the iris, retina, choroid, or optic nerve.¹ The incidence of coloboma in the population is unknown. Colobomas may occur as isolated malformations or as part of a complex multisystem syndrome.¹ Typically, colobomas result from failure of fusion of the embryonic fissure of the optic cup anywhere along the line extending from the optic disc to the inferonasal border of the pupillary margin. For the purposes of the present paper we shall define the term coloboma to include lesions affecting the optic nerve (ranging from optic disc dysplasia to optic nerve aplasia), retina, choroid, and iris, but not the eyelids. Orbital

cysts and microphthalmia are also considered to be part of the spectrum of colobomatous defects resulting from failure of fusion of the embryonic fissure in the optic cup. Rarely, colobomas are reported in association with other developmental anomalies. For example, renal-coloboma syndrome (MIM 120330) is a rare autosomal dominant syndrome involving optic nerve coloboma in association with renal anomalies.²⁻⁷ Other syndromes that may also represent coloboma association syndromes include CHARGE association (coloboma, heart defects, choanal atresia, retarded growth or development, genital hypoplasia, and ear anomalies),⁸ Joubert related syndromes such as Dekaban syndrome (chorioretinal coloboma with cerebellar vermis aplasia and polycystic kidneys),⁹ COACH syndrome (cerebellar vermis hypo/aplasia, oligophrenia, congenital ataxia, coloboma, hepatic fibrosis),¹⁰ acro-reno-ocular syndrome,¹¹ Sorsby syndrome,¹² branchio-oculo-facial syndrome,¹³ cat eye syndrome,¹⁴ and 3C syndrome.¹⁵

We have previously documented mutations of the PAX2 gene in 11 subjects with renal-coloboma syndrome, including a family with autosomal dominant optic nerve coloboma (malformation of the optic nerve), renal anomalies, and vesicoureteral reflux (VUR).¹⁶⁻¹⁹ Recently we found that PAX2 mutations may result in highly variable phenotypes.¹⁹ Since phenotypic variability is a feature of this syndrome we hypothesised that PAX2 mutations may sometimes cause sporadic ocular colobomas in isolation in patients. The specific genes involved in causing sporadic or hereditary colobomas are not known, although hypoplasia or agenesis of the fovea or macula have been reported in some patients with PAX6 mutations.^{20 21}

PAX2 is a member of a multigene family containing a paired box domain that was initially identified in *Drosophila* and subsequently in vertebrates.²² PAX2 expression is critical for development of the urogenital tract, CNS, inner ear, and optic nerve,²³⁻²⁵ as has been reported in mice with kidney and retinal defects (*Krd*),²⁶ *Pax2*^{1Neu} mice,²⁷ and in mice with a *Pax2* gene disruption induced by homologous recombination.^{28 29} To investigate whether PAX2 mutations occur more generally in patients with colobomas, microphthalmia, or retinal anomalies, or in patients with coloboma associated syndromes, we have analysed patient DNA by PCR-SSCP for PAX2 mutations in subjects who have either ocular anomalies alone, a recognised syndrome such as Dekaban

Cancer Genetics
Laboratory,
Department of
Biochemistry,
University of Otago,
PO Box 56, Dunedin,
New Zealand
H E Cunliffe
L A McNoe
T A Ward
M R Eccles

Centre for Human
Genetics, University of
Leuven, Herestraat 49,
Leuven, Belgium
K Devriendt

Department of Human
Genetics, University
Hospital Nijmegen,
Nijmegen, The
Netherlands
H G Brunner

Correspondence to:
Dr Eccles.

Received 26 September 1997
Revised version accepted for
publication 6 March 1998

syndrome, or a loosely defined combination of ocular and urogenital conditions. Following analysis of 99 patients in most or all exons of PAX2, we have identified one new patient with a PAX2 mutation, who had typical renal-coloboma syndrome (optic nerve colobomas, renal anomalies, and vesicoureteric reflux). We did not identify PAX2 mutations in patients with ocular colobomas or microphthalmia in isolation or in patients with ocular colobomas and associated anomalies.

Subjects and methods

CLASSIFICATION OF THE PATIENTS IN THE STUDY

The patients analysed in this study were divided into five categories: (A) patients with typical renal-coloboma syndrome (optic nerve colobomas and renal hypoplasia, with or without vesicoureteric reflux), (B) patients with optic nerve or chorioretinal colobomas, combined with other anomalies, some of whom had a known syndrome, (C) patients with eye anomalies (not optic nerve or chorioretinal coloboma) in combination with other anomalies, who did not fit a known syndrome, (D) patients with isolated renal anomalies, and (E) patients with predominantly ocular anomalies in isolation. Patients in categories A-D are listed in table 1. Patients in category E are listed separately under different headings in table 2. Patients were evaluated by history, physical examination, ophthalmological examination, and renal function tests including routine urine and blood analyses and/or renal ultrasound, intravenous pyelogram, or micturating cystourethrogram.

PCR-SSCP AND DETECTION OF PAX2 MUTATIONS

Genomic DNA from each subject was extracted from peripheral blood using a DNA extraction kit (Promega). Fragments spanning exons 1-12 and the promoter of PAX2 were amplified from genomic DNA using PCR primers flanking the regions of interest (table 3). The primer sequences used for amplifying each exon of PAX2 were based on the genomic sequences flanking each exon.³⁰ Amplification conditions are given in table 3. The PCR products of exons 1-10 and the promoter were labelled by incorporation of ³²P-dCTP in reactions containing 100 ng DNA, 62.5 mmol/l dNTPs, 1 mCi ³²P-dCTP (3000 Ci/mmol), 20 pmol of each primer, reaction buffer (50 mmol/l KCl, 10 mmol/l Tris, pH 8.3, 1.0-3.0 mmol/l MgCl₂), and Hot Tub (Amersham) or Pwo (Boehringer) polymerase. Exons 11 and 12 were labelled by kinasing unlabelled PCR products with polynucleotide kinase (New England Biolabs) and γ ³²P-ATP. The PCR products were electrophoresed on non-denaturing 6% polyacrylamide gels³¹ with 1 × TBE, pH 8.0, buffer to show single strand conformational (SSCP) variants.³² Several conditions of polyacrylamide gel electrophoresis were used, including gels with or without 10% glycerol, and electrophoresis at 25°C or at 4°C. The PCR product from the promoter region was digested with *Sma*I restriction enzyme

(Promega) to yield three fragments of 325, 233, and 232 bp, which were resolved on SSCP gels.

SUBCLONING AND DNA SEQUENCING

PCR products were directly sequenced using α ³²P-dCTP and a cycle sequencing kit (GIBCO-BRL). For subcloning of exon 2 PCR products, mutant and normal alleles of PAX2 were amplified using Pwo DNA polymerase (Boehringer Mannheim), and subcloned into *Eco*RV digested pBluescript II (Stratagene). Positive clones were identified by blue/white selection, and plasmid DNA was isolated using Wizard mini-preps kits (Promega). Sequencing reactions were carried out on plasmid DNA using ³⁵S-dATP and a double stranded sequencing kit (USB).

PAX2 ALLELE DELETION STUDIES

PAX2 mutations could include a deletion of either a part or the whole PAX2 gene from one allele. Such deletions may not necessarily be detected by PCR-SSCP. To investigate whether deletions of the whole PAX2 gene had occurred, heterozygosity of the PAX2 gene was analysed in each patient by amplification of a (CA)_n dinucleotide repeat within intron 9 as previously described.³⁰ Amplification of both alleles was evidence of heterozygosity.

Results

PAX2 MUTATIONAL STUDIES

The results of PCR-SSCP analysis of the PAX2 gene in 99 patients are summarised in tables 1 and 2, along with a summary of the PAX2 mutations which have previously been identified in 11 patients. One patient in this study (patient 985) was found to have an abnormal SSCP pattern in exon 2. No variations in PCR-SSCP patterns were observed in unaffected parental samples of patient 985 (fig 1A) or in 98 other subjects analysed for each exon of PAX2. To identify the PAX2 mutation in patient 985, exon 2 PCR products of patient 985 and his parents were subcloned and sequenced. Sequencing of independently derived mutant alleles from patient 985 identified a deletion of a thymidine nucleotide at position 611 in approximately 50% of clones (fig 1B). Normal alleles were found in the remainder of the clones from patient 985. The observed nucleotide deletion causes a frameshift in exon 2, and would be predicted to terminate the protein within exon 6 encoded sequences (fig 2). The predicted protein would be unable to bind DNA as it would lack much of the paired box DNA binding domain. This mutation would effectively result in haploinsufficiency of PAX2 because the predicted protein from the mutant allele would be non-functional. Fig 2 depicts the location of the mutation in the PAX2 protein and the frameshifted protein which is predicted to result from the mutation, and compares the effect of this mutation to other previously described mutations in PAX2.

CLINICAL REPORT OF PATIENT 985

Patient 985 is the second child born to non-consanguineous Dutch parents. He was

Table 1 Comparison of phenotypes in 39 subjects examined for PAX2 mutations

Patient	Age	Sex	Sporadic/germline	Category (ref)	Phenotype
1	14	M	Germline Family 1	A* (17)	Bilateral optic nerve colobomas, bilateral renal hypoplasia, non-functional right kidney, renal failure, grade IV vesicoureteric reflux, joint laxity
2	10	M	Germline Family 1	A (17)	Bilateral optic nerve colobomas, bilateral renal hypoplasia, grade II vesicoureteric reflux, trace proteinuria, high frequency hearing loss, joint laxity
3	6	M	Germline Family 1	A (17)	Bilateral optic nerve colobomas, bilateral renal hypoplasia, end stage renal failure, renal transplant, grade I vesicoureteric reflux, joint laxity, seizures
4	35	M	Germline Family 1	A (17)	Bilateral optic nerve colobomas, hypertension, mild proteinuria, high frequency hearing loss
430	5	M	Germline Family 2	A (16)	Bilateral optic nerve colobomas, renal tubular atrophy, interstitial fibrosis, proteinuria, absent right kidney, end stage renal failure, renal transplant
431	12.5	M	Germline Family 2	A (16)	Bilateral optic nerve colobomas, proteinuria, renal tubular atrophy, interstitial fibrosis, glomerulosclerosis
TRN	5	M	Sporadic	A (18)	Bilateral optic nerve colobomas, cataracts, renal tubular atrophy, glomerulosclerosis, mild renal dysfunction with proteinuria
579	11	M	Sporadic	A (19)	Bilateral optic nerve colobomas, bilateral renal hypoplasia, proteinuria, progressive end stage renal failure, seizures
656	48	F	Germline Family 3	A (19)	Bilateral optic disc hypoplasia, cataracts, hypertension, proteinuria, bilateral renal hypoplasia, end stage renal failure, renal transplant
657	25	M	Germline Family 3	A (19)	Left optic nerve aplasia, right optic disc hypoplasia with optic pit, chorioretinal coloboma, capsular opacities, microphthalmos, retrobulbar cyst, retardation, microcephaly, bilateral renal hypoplasia, end stage renal failure, renal transplant
2646	20	F	Sporadic	A (19)	Bilateral optic nerve colobomas, bilateral renal hypoplasia, end stage renal failure, focal segmental glomerulosclerosis, right high frequency hearing loss, joint laxity
651	4	F	Sporadic	A (this study)	Bilateral optic nerve colobomas, bilateral renal hypoplasia, impaired renal function, right persistent hyoidal artery
813	12	M	Germline Family 4	A (this study)	Bilateral optic nerve colobomas, acute renal failure at birth, one functional kidney, growth retardation
814	33	F	Germline Family 4	A (this study)	Bilateral optic nerve colobomas, bilateral renal hypoplasia, renal insufficiency from age 18, proteinuria, focal segmental hyalinosis and interstitial sclerosis
816	54	F	Germline Family 4	A (this study)	Bilateral optic nerve colobomas, bilateral renal hypoplasia, renal insufficiency from age 33, proteinuria, end stage renal disease, renal transplant at age 44
906	28	F	Germline Family 5	A (this study)	Bilateral optic nerve colobomas, renal anomalies, renal failure
907	4	M	Germline Family 5	A (this study)	Bilateral optic nerve colobomas, renal anomalies
985	6	M	Sporadic	A (this study)	Bilateral optic nerve colobomas, cystic structure with no functional renal tissue on right side, functional left kidney, no proteinuria, grade II-III reflux in left kidney
518	2.5	M	Sporadic	B† (this study)	Unilateral optic nerve coloboma, renal anomaly, heart defect (double outlet right ventricle)
626	?	?	Sporadic	B (this study)	Joubert related (Dekaban) syndrome, including chorioretinal coloboma and polycystic kidneys
627	?	?	Sporadic	B (this study)	Joubert related (Dekaban) syndrome, including chorioretinal coloboma and polycystic kidneys
630	?	?	Sporadic	B (this study)	Arima syndrome, including coloboma and polycystic kidneys
633	?	?	Sporadic	B (this study)	Arima syndrome, including coloboma and polycystic kidneys
615	?	F	Sporadic	B (this study)	CHARGE association (atypical), including bilateral colobomas of iris and optic nerve
783	2.5	M	Sporadic	B (this study)	Bilateral optic nerve coloboma, macrocystic dysplastic kidneys, severe neurodevelopmental delay, abdominal wall hypoplasia, undescended testes with bilateral hernias, hepatic fibrosis with cholestasis and portal hypertension
806	?	?	Sporadic	B (this study)	Chorioretinal coloboma, renal anomalies, DiGeorge syndrome (thymus aplasia, parathyroid aplasia, aortic arch anomalies)
711	14	M	Germline Family 6	C‡ (this study)	Bilateral iris and retinal colobomas, multicystic non-functional left kidney, left hydronephrosis, normal renal function, intrauterine growth retardation, short stature, dysmorphic features
712	39	M	Germline Family 6	C (this study)	No ocular anomalies, recurrent urinary tract infections, possible vesicoureteric reflux
713	9	F	Germline Family 6	C (this study)	No ocular anomalies, multicystic kidney (resolved by 6 months)
652	?	M	Sporadic	C (this study)	Left iris coloboma, bilateral vesicoureteric reflux, renal failure, bilateral cleft lip and palate
659	37	M	Germline Family 7	C (this study)	Retinitis pigmentosa, lens subluxation, lens coloboma, cataract, right renal agenesis, left chronic interstitial nephritis, left renal hypoplasia, renal transplant age 27
660	39	M	Germline Family 7	C (this study)	Retinitis pigmentosa?, insulin dependent diabetes, globular aspect of lower pole of left kidney
695	14	F	Sporadic	C (this study)	Bilateral iris and retinal coloboma, vesicoureteric reflux, renal failure, renal transplant age 11
697	?	M	Sporadic	C (this study)	Retinopathy, preauricular ear tags, vesicoureteric reflux, cystic kidneys, renal failure, renal transplant
698	?	F	Germline Family 8	C (this study)	Vesicoureteric reflux
699	?	F	Germline Family 8	C (this study)	Cataracts, ataxia, peripheral neuropathy, renal agenesis, vesicoureteric reflux, renal failure, renal transplant
792	2	F	Sporadic	C (this study)	Bilateral microphthalmos, cystic right kidney, left hydronephrosis, right UPJ obstruction, seizures, abnormal EEG
847	8	M	Sporadic	C (this study)	Bilateral microphthalmos, microcorneas, persistent primary hyperplastic vitreous body, orbital cysts, mentally retarded, nephronophthisis, atrophic renal tubules, glomerulosclerosis, hyaline deposits, microtestis, large malformed ears
544	9	M	Germline Family 9	D§ (this study)	Unilateral renal agenesis

*Renal-coloboma syndrome.

†Optic nerve coloboma associated or chorioretinal coloboma associated syndrome.

‡Syndrome with renal and eye abnormalities atypical of renal-coloboma syndrome.

§Kidney abnormalities only. ND = not done. UN = uninformative. P = promoter.

born after 36 weeks' gestation, with a birth weight of 2430 g. At 6 weeks he was evaluated for failure to thrive, at which time a raised creatinine level (121 $\mu\text{mol/l}$) was found. Renal ultrasound showed a cystic structure on the right side, with no recognisable kidney. The left

kidney appeared normal, except for dilatation of the upper pole. A renal scan showed a functional left kidney with delay in excretion, but no functional renal tissue was present on the right side. A voiding cystogram showed a normal bladder with grade II-III reflux on the left side.

Table 1 Continued

Karyotype	Heterozygosity of PAX2	Exons analysed	Mutation
Normal	Heterozygous	1-5	Exon 5
ND	Heterozygous	1-5	Exon 5
ND	Heterozygous	1-5	Exon 5
ND	Heterozygous	1-5	Exon 5
Normal	Heterozygous	1-5	Exon 2
Normal	Heterozygous	1-5	Exon 2
t(10;13)(q24; q12.3)	Heterozygous	ND	Translocation
Normal	Heterozygous	1-12	Exon 2
ND	Heterozygous	1-4, 7-12	Exon 2
Normal	Heterozygous	1-10, 12	Exon 2
Normal	Heterozygous	1-10	Exon 2
ND	Heterozygous	1-12, P	No
Normal	Heterozygous	1-12, P	No
Normal	Heterozygous	1-10, 12	No
Normal	Heterozygous	1-10, 12	No
ND	ND	1-12, P	No
ND	ND	1-12, P	No
ND	Heterozygous	2, 5, 8, 10-12, P	Exon 2
ND	Heterozygous	1-10	No
ND	ND	1-4, 6-12, P	No
ND	ND	1-12, P	No
ND	ND	1-11	No
ND	ND	1-12, P	No
ND	ND	1-10	No
ND	UN	1-10	No
ND	Heterozygous	1-12, P	No
ND	UN	1-10	No
ND	UN	1-9	No
ND	UN	1-9, 12, P	No
Normal	UN	1-10, 12, P	No
ND	ND	1-8, 10, 12	No
ND	ND	1-12, P	No
ND	ND	1-12, P	No
ND	ND	1-10	No
ND	ND	1-10	No
ND	ND	1-9	No
ND	ND	1-12, P	No
Normal	ND	1-12, P	No
ND	Heterozygous	1-11	No

An intravenous pyelogram showed a visible left kidney without obvious abnormalities. Urine analysis was normal with no proteinuria and occasional leucocytes. At 3 months of age the patient had a right nephrectomy and vesicosotomy. Ocular abnormalities included a convergent squint and abnormal optic nerves with colobomas. The fundi were otherwise normal, as were lens tissues and anterior chambers.

Additional investigations, including MRI scan, x ray of the spine, ECG, BAEP, and VEP all yielded normal results. His general health and development have been normal, except for recurrent parotitis, which was attributed to Furadantin maintenance therapy.

Discussion

Five different PAX2 mutations have been identified in 12 subjects with renal-coloboma syndrome¹⁶⁻¹⁹ (this study). Most people with PAX2 mutations have been found to suffer from optic nerve colobomas, renal hypogenesis, and renal dysfunction, although some patients had one or more additional anomalies, including vesicoureteric reflux (VUR), high frequency hearing loss, CNS anomalies, other eye abnormalities, and joint laxity (table 1). The patient with a PAX2 mutation in this study (patient 985) presented with bilateral optic nerve colobomas, a functional left kidney, a cystic structure in place of the right kidney, and vesicoureteric reflux, and was found to have a deletion of a thymidine nucleotide at position 611 in exon 2 of PAX2.¹⁷ The identification of a PAX2 mutation in this patient concurs with the identification of a PAX2 mutation in a family with optic nerve colobomas, vesicoureteric reflux, and renal anomalies in a previous study,¹⁷ and suggests that in a small sample of patients the position of the mutation within the PAX2 gene does not necessarily correlate with the occurrence of vesicoureteric reflux; patient 985 in this study had a mutation in exon 2, while the three sibs in the previous study, who had vesicoureteric reflux,¹⁷ were found to have a mutation in exon 5 of PAX2.

The phenotype associated with PAX2 mutations is highly variable, even in patients with identical mutations.¹⁹ In view of the marked variability in expression of the PAX2 mutant phenotype, we have hypothesised that PAX2 mutations may cause eye, renal, or ureteric abnormalities in isolation. In this study we examined 99 patients with either colobomas or microphthalmia in isolation or colobomas and associated anomalies for PAX2 mutations by PCR-SSCP analysis. We found that novel PAX2 sequence variants were uniformly absent in these patients, except for the identification of a PAX2 mutation in patient 985. A full list of the phenotypic abnormalities found in 110 patients analysed for PAX2 mutations, including previously reported patients, is given in tables 1 and 2. The identification of the spectrum of phenotypic features in patients who have renal-coloboma syndrome will be clinically useful as a basis for assessing which patients to screen for PAX2 mutations.

We have previously reported that PAX2 mutations are absent in patients with isolated familial vesicoureteric reflux.^{33, 34} These findings suggest that PAX2 mutations may not be found in patients with ureteric anomalies in isolation. However, it remains to be determined whether PAX2 mutations will be associated with isolated renal abnormalities. PAX2 mutations are associated with renal hypoplasia, tubular atrophy, glomerulosclerosis, progressive renal failure, and vesicoureteric reflux

Table 2 Absence of PAX2 mutations in 71 subjects or families with predominantly eye abnormalities

Phenotype	Inheritance	No of patients analysed (no of families)
Optic nerve hypoplasia (or coloboma)	Sporadic	5
Optic nerve hypoplasia (or coloboma)	Inherited	2 (1)
Optic nerve coloboma with cataract	Sporadic	1
Bilateral optic nerve colobomas with dysmorphic features	Sporadic	1
Bilateral optic nerve colobomas (with hypospadias in 2 subjects)	Inherited	4 (1)
Microphthalmos	Sporadic	26
Microphthalmos	Inherited	13 (6)
Microphthalmos with optic nerve hypoplasia	Sporadic	1
Microphthalmos with iris, choroid, retina and optic disc coloboma (and VUR in 1 subject)	Inherited* (mother had only pigment deposition on retina in line with colobomatous cleft)	3 (1)
Microphthalmos with bilateral iris and retinal colobomas and pupil coloboma	Sporadic*	1
Unilateral chorioretinal coloboma (plus extreme contralateral microphthalmos and PUJ obstruction in daughter)	Inherited*	2 (1)
Aniridia	Sporadic	6
Aniridia	Inherited	0
Bilateral aniridia with optic nerve and foveal hypoplasia	Sporadic	1
Iris coloboma with bilateral retinal hypoplasia	Inherited*	1 (1)
Bilateral pupil coloboma	Sporadic	1
Optic neuropathy	Sporadic	2
Hypoplasia of ciliary body, choroid, retina, and optic nerve	Sporadic*	1

*Subjects analysed who were heterozygous for a polymorphism in PAX2.

Table 3 PAX2 PCR-SSCP primer sequences and reaction conditions

Exon	Sequence	Mg conc	Tm	Product size (bp)
PF†	5' GGG CTC CAG CGC TGG CGA ATC ACA 3'			
PR‡	5' CGC CGC CGT GTC GCC TCT CAA ACT 3'	1.5	65	791
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' CCC CTC CCT GCA AAC CAC 3'			
10R	5' CGC TGT GAG GGC CAT GAC 3'	2.0	57	150
11F	5' GCA GGC GTC ACA TCC CCA CTC 3'			
11R	5' CCG GCC ACC AGG TGG CGT A 3'	1.5	52	148
12F	5' ATG TGG AGG CCG AAG CTG 3'			
12R	5' TCT GAC CCA GCC CAT TCT TCT 3'	1.0	60	163

*P, promoter.

†F, forward primer.

‡R, reverse primer.

(table 1). One of us (KD) has recently identified a PAX2 missense mutation in a large pedigree presenting with renal hypoplasia and mild ocular anomalies (submitted). All affected members had renal involvement with only mild optic disc anomalies, which in most cases did not affect visual acuity. These findings emphasise the importance of an eye examination in patients presenting with renal hypoplasia.

Mutations in PAX2 appear predominantly to cause optic nerve abnormalities and renal hypoplasia in patients with renal-coloboma syndrome. It is not known what genes might be

responsible for optic nerve colobomas in isolation, or for the variable association of colobomas and urogenital anomalies in renal-coloboma-like syndromes. Several genes could be candidates, including the CHX10 gene which has been found to cause microphthalmia in mice,³⁵ or the putative gene for cat eye syndrome on chromosome 22, which has yet to be identified.

That we did not detect PAX2 mutations in 98 patients who had either ocular abnormalities in isolation or eye defects with associated abnormalities suggests that PAX2 mutations do not cause colobomas in isolation, microphthalmia or retinal anomalies, or syndromes of coloboma, renal anomalies, and atypical associated features. In agreement with these results a family with dominant inheritance of optic pits was analysed for PAX2 mutations by SSCP in exons 1-10 of PAX2 and was not found to have PAX2 sequence variants.³⁶ Included among the patients analysed in this study were two patients with a subtype of Joubert syndrome, referred to as Dekaban syndrome⁹ (chorioretinal coloboma with cerebellar vermis aplasia and polycystic kidneys) (patients 626 and 627), and also two patients with Arima syndrome³⁷ (blindness, cerebellar vermis agenesis, polycystic kidneys, hepatic fibrosis) (patients 630 and 633). Several other syndromes, for example acro-renal-ocular syndrome,¹¹ branchio-oculo-facial syndrome,¹³ and cat eye syndrome,¹⁴ may include optic nerve colobomas as part of the phenotype, but we have not examined such patients for PAX2 mutations.

In this study three families had a syndrome that was considered to be identical to renal-coloboma syndrome (patients 651, 813, 814, 816, 906, and 907), yet PAX2 mutations were not identified in these patients. One explanation for the lack of PAX2 mutations is that there is a low level of PCR-SSCP sensitivity in our screening procedure, and that certain types of mutations are not being detected.³⁸ However, our experience with the PCR-SSCP method has shown that it is sufficiently sensitive (85-100% detection rate) to identify polymorphisms in DNA sequences with a GC content ranging from 55 to 68% (HEC, unpublished data). This level of detection of polymorphisms by PCR-SSCP is in agreement with observations made by others.³⁹ Alternative explanations as to why only seven out of 10 families with renal-coloboma syndrome were identified as having PAX2 mutations are that the mutations were in sequences that we have not yet analysed, or that there are heterogeneous causes for the association of optic nerve colobomas and renal anomalies. Mutations in genes either affecting the expression of PAX2 (giving rise to insufficient PAX2 expression) or in downstream targets of the PAX2 protein might be predicted to give rise to a syndrome with features closely resembling that of renal-coloboma syndrome.

We conclude from this study that PAX2 mutations are unlikely to be common in patients with ocular colobomas in isolation or in patients with ocular colobomas and associated anomalies. However, patients with typical

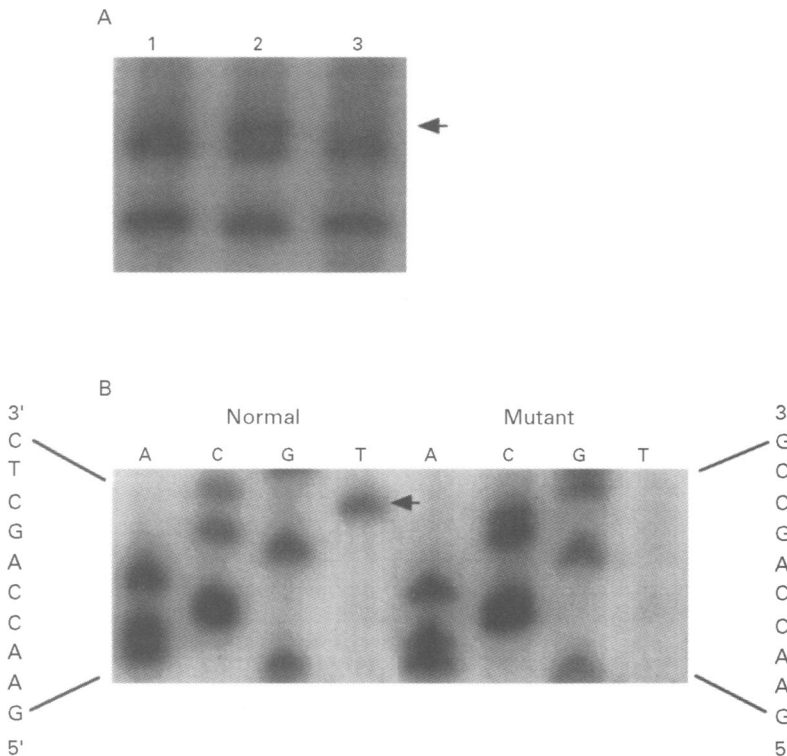


Figure 1 Identification of the mutation in exon 2 of PAX2. (A) SSCP analysis of exon 2. The primers and conditions for amplification are described in table 3. Lane 1, mother's DNA. Lane 2, affected subject's DNA (patient 985). Lane 3, father's DNA. An additional band is present in lane 2 (arrow) in DNA from the affected subject. The pattern observed in the mother's and father's DNA is normal. (B) Sequence of the normal and mutant alleles from patient 985. Seven independent clones in pbluescript were identified that contained an identical mutation, and a further seven clones from the affected patient's DNA contained normal PAX2 sequence. A single base deletion (arrow) was observed in the affected subject's DNA.

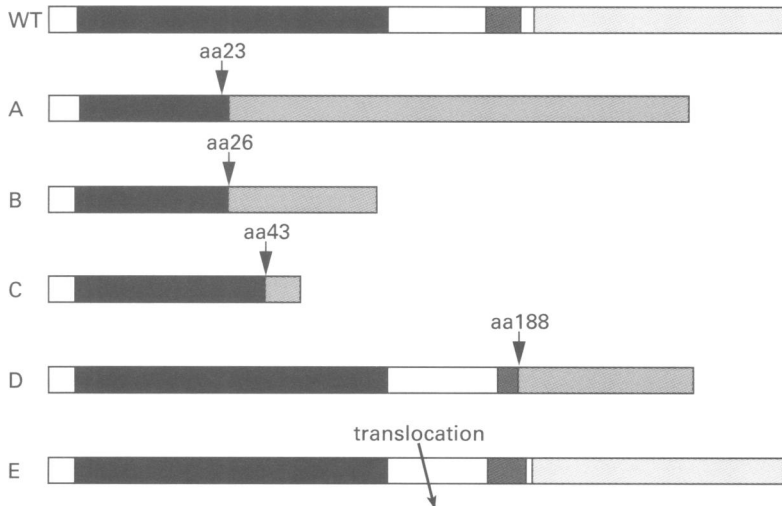


Figure 2 Depiction of the location of mutations which have been identified to date within PAX2 and the effect of these mutations on the predicted PAX2 protein products. The boxed segments represent the protein domains. The conserved paired box domain (black portion), the octapeptide sequence (dark grey portion), and the transactivation domain (light grey portion) are shown. Frameshifted sequences in the mutated protein are depicted by mid grey portions. WT is the normal wild type protein. A is the mutation identified in the present study, with a single base deletion at amino acid 23 which results in a frameshift and a truncated protein. B, C, D, and E depict the predicted protein of previously identified mutations.^{16, 19} The mutation in B is a single base insertion at amino acid position 26.^{16, 19} The mutation in C is a 22 base deletion at amino acid position 43.¹⁹ The mutation in D is a single base insertion at amino acid position 188 in the octapeptide domain.¹⁷ All of these point mutations are predicted to cause a frameshift and truncated protein product. Note, that the predicted proteins in A, B, and C lack much of the paired DNA binding domain (black), as well as the octapeptide domain and COOH-terminal portions of PAX2 protein, whereas the protein in D lacks only the octapeptide and remaining COOH-terminal portions. The mutation in E is a translocation (10;13)(q24;q12.3) with a breakpoint in the middle of the PAX2 gene,¹⁸ probably within either intron 3 or 4, which would result in an abnormal, and possibly truncated protein.

renal-coloboma syndrome are very likely to have PAX2 mutations.

We gratefully acknowledge Drs R McInnes, A Levin, R J M Gardner, E Haan, P Bitoun, I Gupta, J P Fillastre, R Nelson, L Market, D Clark, J Pellegrino, R Koenekoop, A Schinzel, and Y Pirson for the provision of patient samples for analysis in this study. We also thank Drs. H Cruysberg, L Monnens, and R De Boer for additional clinical information on the affected patient. This research was funded by the Health Research Council of New Zealand, the Cancer Society of New Zealand, and the New Zealand Lotteries Grants.

- Pagon RA. Ocular coloboma. *Surv Ophthalmol* 1981;25:223-36.
- Reiger G. Zum krankheitsbild der Handmannschen sehnerven-anomalie: windenbluten- (Morning Glory) syndrom? *Klin Mbl Augenheilkd* 1977;170:697-706.
- Karcher H. Zum Morning Glory syndrom. *Klin Mbl Augenheilkd* 1979;175:835-40.
- Weaver RG, Cashwell LF, Lorentz W, Whiteman D, Geisinger KR, Ball M. Optic nerve coloboma associated with renal disease. *Am J Med Genet* 1988;29:597-605.
- Bron AJ, Burgess SE, Awdry PN, Oliver D, Arden G. Papillo-renal syndrome. An inherited association of optic disc dysplasia and renal disease. Report and review of the literature. *Ophthalm Paediatr Genet* 1989;10:185-98.
- Legros Y, Carrenard JR, Cortes FM, Sanchez JM. Syndrome de "ojo de gato" con agenesia renal derecha. Aportacion de un caso y revision de la literatura. *An Esp Pediatr* 1992;36:317-19.
- 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* 1995;59:204-8.
- Pagon RA, Graham JM Jr, Zonana J, Yong SL. Coloboma, congenital heart disease, and choanal atresia with multiple anomalies: CHARGE association. *J Pediatr* 1981;99:223-7.
- Saraiva JM, Baraitser M. Joubert syndrome: a review. *Am J Med Genet* 1992;43:726-31.
- Verloes A, Lambotte C. Further delineation of a syndrome of cerebellar vermis hypo/aplasia, oligophrenia, congenital ataxia, coloboma, and hepatic fibrosis. *Am J Med Genet* 1989;32:227-32.
- Halal F, Homsy M, Perreault G. Acro-renal-ocular syndrome: autosomal dominant thumb hypoplasia, renal ectopia, and eye defect. *Am J Med Genet* 1984;17:753-62.
- Sorsby A. Congenital coloboma of the macula, together with an account of the familial occurrence of bilateral macular coloboma in association with apical dystrophy of hands and feet. *Br J Ophthalmol* 1935;19:65-90.
- Richardson E, Davison C, Moore A. Colobomatous microphthalmia with midfacial clefting: part of the spectrum of branchio-oculo-facial syndrome? *Ophthalm Genet* 1996;17:59-65.
- Schinzel A, Schmid W, Fraccaro M, et al. The 'cat eye syndrome': dicentric small marker chromosome probably derived from a 22 (tetrasomy 22pter;q11) associated with a characteristic phenotype. Report of 11 patients and delineation of the clinical picture. *Hum Genet* 1981;57:148-58.
- Marles SL, Chodirker BN, Greenberg CR, Chudley AE. Evidence for Ritscher-Schinzel syndrome in Canadian native Indians. *Am J Med Genet* 1995;56:343-50.
- Sanyanusin P, McNoe LA, Sullivan MJ, Weaver RG, Eccles MR. Mutation of PAX2 in two siblings with renal-coloboma syndrome. *Hum Mol Genet* 1995;4:2183-4.
- Sanyanusin P, Schimmenti LA, McNoe LA, et al. Mutation of the PAX2 gene in a family with optic nerve colobomas, renal anomalies and vesicoureteral reflux. *Nat Genet* 1995;9:358-64.
- Narahara K, Baker E, Ito S, et al. 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* 1997;34:213-16.
- Schimmenti LA, Cunliffe HE, McNoe LA, et al. Further delineation of the renal-coloboma syndrome in patients with extreme variability of phenotype and identical PAX2 mutations. *Am J Hum Genet* 1997;60:869-78.
- Azuma N, Nishina S, Yanagisawa H, Okuyama T, Yamada M. PAX6 missense mutation in isolated foveal hypoplasia. *Nat Genet* 1996;13:141-2.
- Martha A, Strong LC, Ferrell RE, Saunders GF. Three novel aniridia mutations in the human PAX6 gene. *Hum Mutat* 1995;6:44-9.
- Strachan T, Read AP. PAX genes. *Curr Opin Genet Dev* 1994;4:427-38.
- Dressler GR, Deutsch U, Chowdhury K, Nornes HO, Gruss P. Pax2, a new murine paired-box-containing gene and its expression in the developing excretory system. *Development* 1990;109:787-95.
- Nornes HO, Dressler GR, Knapik EW, Deutsch U, Gruss P. Spatially and temporally restricted expression of Pax2 during murine neurogenesis. *Development* 1990;109:797-809.
- Eccles MR, Wallis LJ, Fidler AE, Spurr NK, Goodfellow PJ, Reeve AE. Expression of the PAX2 gene in human fetal kidney and Wilms' tumor. *Cell Growth Diff* 1992;3:279-89.
- Keller SA, Jones JM, Boyle A, et al. Kidney and retinal defects (Krd), a transgene-induced mutation with a deletion of mouse chromosome 19 that includes the Pax2 locus. *Genomics* 1994;23:309-20.
- Favor J, Sandaluche R, Neuhauser-Klaus A, et al. The mouse Pax2^{1Ncu} mutation is identical to a human PAX2

- mutation in a family with renal-coloboma syndrome and results in developmental defects of brain, ear, eye and kidney. *Proc Natl Acad Sci USA* 1996;93:13870-5.
- 28 Torres M, Gomez-Pardo E, Dressler GR, Gruss P. *Pax2* controls multiple steps of urogenital development. *Development* 1995;121:4057-65.
 - 29 Torres M, Gomez-Pardo E, Gruss P. *Pax2* contributes to inner ear patterning and optic nerve trajectory. *Development* 1996;122:3381-91.
 - 30 Sanyanusin P, Norrish JH, Ward TA, Nebel A, McNoe LA, Eccles MR. Genomic structure of the human *PAX2* gene. *Genomics* 1996;35:258-61.
 - 31 Sambrook J, Fritsch EF, Maniatis T. *Molecular cloning: a laboratory manual*. 2nd ed. New York: Cold Spring Harbor Laboratory Press, 1989.
 - 32 Orita M, Suzuki Y, Sekiya T, Hayashi K. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics* 1989;5:874-9.
 - 33 Choi KL, McNoe LA, French MC, Guilford PJ, Eccles MR. Absence of *PAX2* gene mutations in patients with primary familial vesicoureteric reflux. *J Med Genet* 1998;35:338-9.
 - 34 Eccles MR, Bailey RR, Abbott GD, Sullivan MJ. Unraveling the genetics of vesicoureteric reflux: a common familial disorder. *Hum Mol Genet* 1996;5:1425-9.
 - 35 Burmeister M, Novak J, Liang MY, et al. Ocular retardation mouse caused by *Chx10* homeobox null allele: impaired retinal progenitor proliferation and bipolar cell differentiation. *Nat Genet* 1996;12:376-84.
 - 36 Stefko ST, Campochiaro P, Wang P, Li Y, Zhu D, Traboulsi E. Dominant inheritance of optic pits. *Am J Ophthalmol* 1997;124:112-13.
 - 37 Matsuzaka T, Sakuragawa N, Nakayama H, Sugai K, Kohno Y, Arima M. Cerebro-oculo-hepato-renal syndrome (Arima's syndrome): a distinct clinicopathological entity. *J Child Neurol* 1986;1:338-46.
 - 38 Axton R, Hanson I, Danes S, Sellar G, van Heyningen V, Prosser J. The incidence of *PAX6* mutation in patients with simple aniridia: an evaluation of mutation detection in 12 cases. *J Med Genet* 1997;34:279-86.
 - 39 Hayashi K. PCR-SSCP: a simple and sensitive method for detection of mutations in the genomic DNA. *PCR Methods and Applications* 1991;7:34-8.