

Phenotypic Variability and Asymmetry of Rieger Syndrome Associated with *PITX2* Mutations

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PURPOSE. Rieger syndrome is an autosomal dominant condition characterized by a variable combination of anterior segment dysgenesis, dental anomalies, and umbilical hernia. To date, reports have shown mutations within the *PITX2* gene associated with Rieger syndrome, iridogoniodysgenesis, and iris hypoplasia. The purposes of this study were to determine the range of expression and intrafamilial variability of *PITX2* mutations in patients with anterior segment dysgenesis.

METHODS. Seventy-six patients with different forms of anterior segment dysgenesis were classified clinically. DNA was obtained and screened by means of polymerase chain reaction (PCR)-single-stranded conformation polymorphism (SSCP) and heteroduplex analysis followed by direct sequencing.

RESULTS. Eight of 76 patients had mutations within the *PITX2* gene. Anterior segment phenotypes show wide variability and include a phenocopy of aniridia and Peters', Rieger, and Axenfeld anomalies. Mutations include premature terminations and splice-site and homeobox mutations, confirming that haploinsufficiency the likely pathogenic mechanism in the majority of cases.

CONCLUSIONS. There is significant phenotypic variability in patients with *PITX2* mutations, both within and between families. Developmental glaucoma is common. The umbilical and dental abnormalities are highly penetrant, define those at risk of carrying mutations in this gene, and guide mutation analysis. In addition, there is a range of other extraocular manifestations. (*Invest Ophthalmol Vis Sci.* 2000;41:2456-2460)

Rieger syndrome is an autosomal dominant condition characterized by ocular anterior segment dysgenesis and dental and umbilical abnormalities.¹⁻³ Classically, the ocular features include posterior embryotoxon, iris stromal hypoplasia, corectopia, and polycoria associated with a high risk of developmental glaucoma. After the observation of Rieger syndrome in patients with chromosomal rearrangements, a locus on chromosome 4q25 was identified and mutations found within the *PITX2* gene.⁴⁻⁷ The gene consists of four exons and encodes a homeo domain characteristic of the bicoid-related proteins. In addition to Rieger syndrome, *PITX2* mutations have been described in autosomal dominant iris

hypoplasia and iridogoniodysgenesis type 2.^{8,9} Heterogeneity among families with Rieger syndrome has been confirmed with the description of an additional locus situated at 13q14.¹⁰

There is a wide variety of anterior segment dysgenesis phenotypes. These include aniridia and Peters', Rieger, and Axenfeld anomalies. For the genes identified that underlie inherited cases, both phenotypic variability of mutations at the same locus and locus heterogeneity among similar phenotypes are described¹¹⁻¹⁴ (Table 1). Although several mutations have been described at the *PAX6* locus,¹⁵ uncertainty remains about the severity and variability of mutations in other genes that give rise to anterior segment dysgenesis. To address this question, we screened the *PITX2* gene in a panel of 76 unrelated patients with anterior segment dysgenesis phenotypes and defined the phenotypic range among eight families with identified mutations.

METHODS

Clinical Details

All individuals were ascertained on the basis of a history of anterior segment dysgenesis, according to guidelines approved by the North-West Region Ethics Committee and the Declaration of Helsinki. Proband underwent a complete eye examination, including slit lamp biomicroscopy, applanation tonometry, and dilated fundus examination. Blood samples were obtained, and DNA was extracted using conventional methods.¹⁶

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TABLE 1. Genetic Localizations of Genes Involved in Isolated Anterior Segment Dysgenesis Syndromes

Chromosomal Location	Gene	Phenotype
11p13	<i>PAX6</i>	Aniridia, Peters anomaly, anterior polar cataract
4q25	<i>PITX2</i>	Axenfeld-Rieger syndrome, iridogoniodysgenesis syndrome (IRID2), iris hypoplasia with early glaucoma (IGHA)
10q25	<i>PITX3</i>	Anterior segment mesenchymal dysgenesis; (ASMD), anterior polar cataract
6p25	<i>FKHL7</i>	Axenfeld-Rieger syndrome, iridogoniodysgenesis anomaly (IRID1)
6p25	Not identified	Glaucoma and iridogoniodysgenesis, iridogoniodysgenesis anomaly (IRID1)
13q14	Not identified	Rieger syndrome

PCR Analysis

All four *PITX2* exons were amplified using primers, as previously reported.⁷ Genomic DNA (50 ng) was suspended in a 20 μ l reaction containing 10 picomoles each of the forward and reverse primers; 5 mM each of dCTP, dGTP, dTTP, and dATP; 1 \times PCR buffer (containing 10 mM Tris-HCl [pH 8.3], 50 mM KCl, 1–1.5 mM MgCl₂, and 0.1% gelatin), overlaid with mineral oil. The samples were heated to 96°C for 10 minutes (denaturation) then cooled to 51°C (annealing), and 0.15 units *Taq* DNA polymerase was added. The samples were processed in the following conditions: 92°C for 30 seconds, 51°C for 30 seconds, and 72°C for 30 seconds for 35 cycles and 72°C for 10 minutes. To the amplified products an equal volume of formamide stop solution was added. Gels were run at 350 V overnight at 4°C and silver stained according to standard protocols. In cases in which an SSCP shift was observed, direct sequencing of PCR products was performed using a dye terminator cycle sequencing kit (Perkin-Elmer–Applied Biosystems, Warrington, UK) using a fluorescent sequencer (ABI 373), according to the manufacturer's instructions. For insertion and deletion mutations, abnormally migrating SSCP bands were purified and reamplified before sequencing. All SSCP abnormalities were sought (and were absent) in 100 normal control chromosomes.

RESULTS

PITX2 mutations were found in 8 (11%) of 76 patients with anterior segment dysgenesis phenotypes. Of the eight cases, six are familial, whereas two (families 5 and 6) are sporadic (Fig. 1). Families 1 and 2 have been reported previously.^{16,18} The anterior segment phenotypes included diagnoses of aniridia and Peters, Rieger, and Axenfeld anomalies. None of the mutation carriers had normal findings in an ocular examination. Of 14 individuals for whom data were available, 8 had a history of glaucoma. The main clinical findings and mutations are listed in Table 2, and examples of the anterior segment findings shown in Figure 2.

Of the eight mutations described, which were all different, two lay within the homeodomain (families 6 and 8). In family 6 (analyzed simultaneously by others: J. Murray, personal communication, November 1999) the A-to-G transition at nucleotide 845 results in the replacement of lysine codon 88 by glutamine at position 50 within helices 3 to 4 of the homeodomain. In family 8 the mutation is a C-to-T substitution of nucleotide 851, which results in the replacement of an arginine

by a cysteine at residue 90. There were two splice-site mutations in families 1 and 3. These mutations were identified at positions –1 of the 3' splice site of intron 2 (family 3) and –2 of the 3' splice site of intron 3 (family 1), which lies within the homeodomain. Finally, there were four nonsense mutations in families 2, 4, 5, and 7. Examples of the sequencing results are given in Figure 3.

DISCUSSION

We have screened the *PITX2* gene by SSCP-heteroduplex analysis in 76 unrelated patients with a wide variety of anterior segment dysgenesis phenotypes including Peters' anomaly, Rieger syndrome and anomaly, anterior segment mesenchymal dysgenesis, aniridia (without *PAX6* mutations), Axenfeld anomaly, and sclerocornea. Among these, a wide variety of nonocular manifestations, including cardiac anomalies, Peters' plus syndrome, and skeletal abnormalities were noted, reflecting the observation that more than 70 of the syndromes included in the London dysmorphology database include anterior segment malformation.¹⁹

Fifteen individuals within eight families carried *PITX2* mutations and had signs of anterior segment dysgenesis. The ocular manifestations were widely variable (Table 2, Fig. 2). In one family (family 3) gross iris hypoplasia resulted in an initial diagnosis of aniridia in one affected individual. This patient had no evidence of foveal hypoplasia and did not have nystagmus and therefore did not have the classic manifestations of true

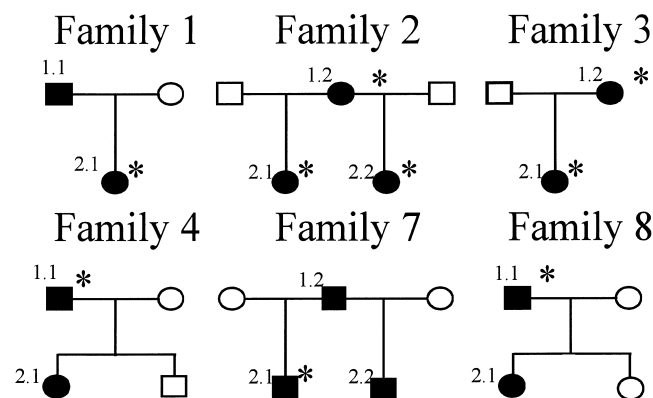


FIGURE 1. Pedigrees of familial cases described in this study. Asterisks represent individuals available for DNA analysis.

TABLE 2. Clinical Findings and Mutations in Families with *PITX2* Mutations

Family	Patient	Anterior Segment Phenotype	Glaucoma	Dental Abnormalities	Umbilical Abnormalities	Additional Findings	Visual Acuity	Mutation
1	1.1	NK	+	+	NK		NK	Not tested
1	2.1	L: Rieger anomaly, anterior polar cataract R: Peters anomaly	- -	+	+	Mild left foveal hypoplasia	L 6/18; R LP	Ivs 3 (-2); A→T
2	1.2	L and R: Iris hypoplasia, PAS	-	+	NK		L 6/9; R 6/12	C ins 1083
2	2.1	L: Peters anomaly R: Iris hypoplasia, Axenfeld anomaly	- -	+	NK		L CF; R 6/9	C ins 1083
2	2.2	L and R: Rieger anomaly	-L, +R	-	NK		L 6/9; R NLP	C ins 1083
3	1.2	L and R: Severe iris hypoplasia imulating aniridia	+	+	+		L 6/12; R 6/9	Ivs 2 (-1), G→C
3	2.1	L and R: Rieger anomaly	+	+	+	Anisometropia, joint hypermobility, anteriorly placed anus	L 6/9; R 6/24	Ivs 2 (-1), G→C
4	1.1	L and R: Rieger anomaly	+	+	+		L NLP; R CF	A del 939
4	2.1	L and R: Mild iris hypoplasia, PAS	-	+	+		Good	Not tested
5	1	L and R: Rieger anomaly	+	+	+	Meckel's diverticulum	L 6/5; R 6/9-2	1235-1236, TA→AAG
6	1	L and R: Rieger anomaly	NK	+	+		NK	A 845 T
7	1.2	NK	+	+	+		Blind	NK
7	2.1	L and R: Rieger anomaly, PAS	-	+	+	Imperforate anus, pectus deformity, finger pulp abnormality	L 6/6; R 6/12	AA del 868-869
7	2.2	NK	NK	+	+		NK	NK
8	1.1	L and R: Rieger anomaly	-	+	+	Cleft palate, learning difficulties	Good	C 851 T
8	2.1	NK	+	+	NK	Cleft uvula, edentulous	NK	Not tested

L, left eye; R, right eye; NK, not known; ivs, intervening sequence; ins, insertion; PAS, peripheral anterior synechia; CF, count fingers; LP, light perception; NLP, no light perception.

aniridia. However, this is the first description of a phenocopy of aniridia in a patient with a proven *PITX2* mutation.

Other anterior segment phenotypes included unilateral Peters' anomaly, Rieger anomaly, and Axenfeld anomaly-iris

hypoplasia. That there is such a wide overlap of the phenotypic features between eyes of the same patient, within and between families, suggests that the clinically and morphologically defined ocular phenotypes do not fall within biologic or

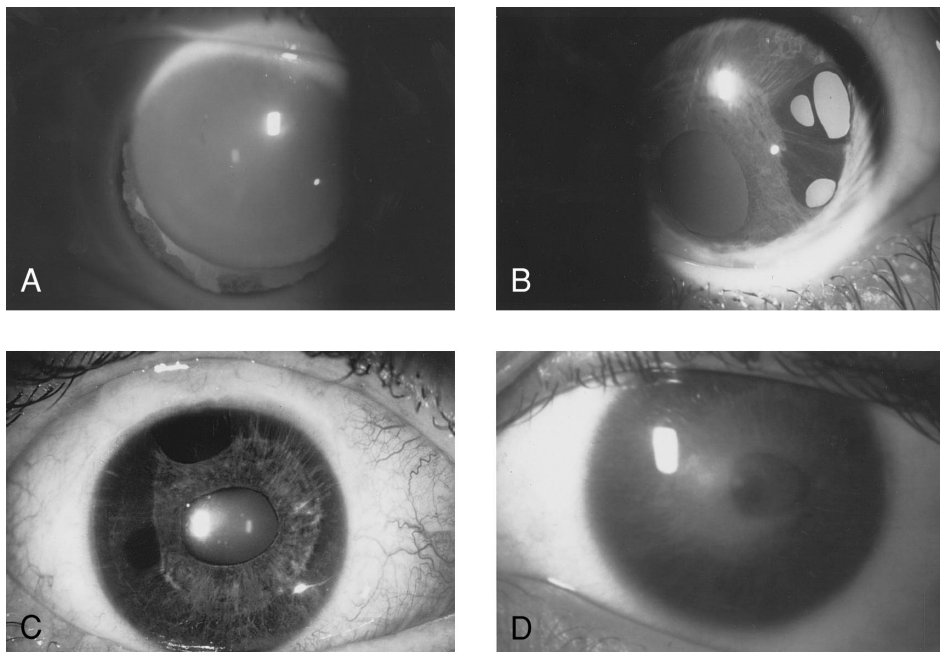


FIGURE 2. Ocular features of patients described in this study. (A) Severe iris hypoplasia, simulating aniridia (case 1, family 3). In transillumination, there is total absence of iris with lens and zonules clearly visible. Pigment deposition on peripheral corneal endothelium is present inferiorly. (B) Rieger anomaly (case 2, family 3). Polycoria and corectopia with iris hypoplasia superiorly. (C) Rieger anomaly (family 5) with corectopia and widespread iris hypoplasia and full-thickness iris defects at 9 and 12 o'clock. (D) Peters' anomaly (case 2, family 2) with central corneal opacity and corectopia.

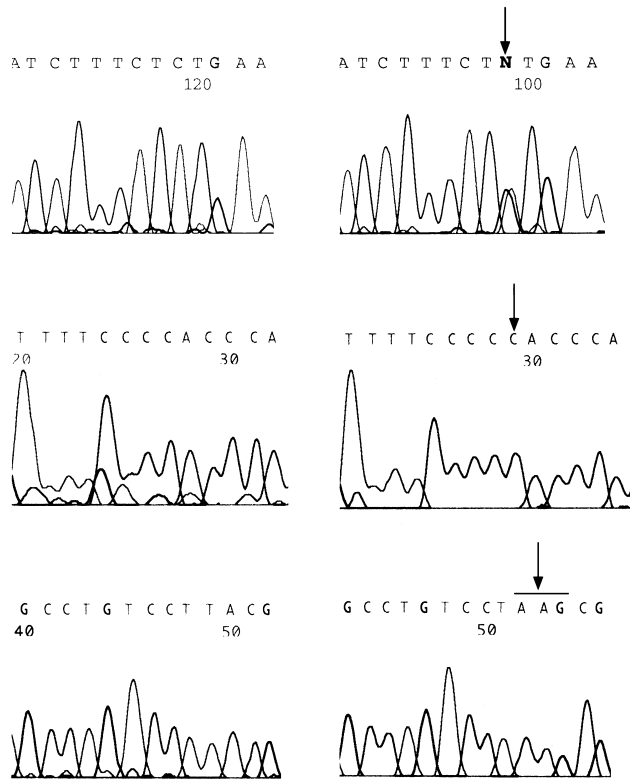


FIGURE 3. Examples of sequencing of mutations in *PITX2*. *Left:* Normal control. *Right Patient.* *Top:* Sequence of 5' of exon 3 (reverse) direction in heterozygous state in family 3. G→C substitution in -1 position of 3' end of intron 2. *Middle:* Sequence of purified mutant allele, exon 4, family 2 showing C ins 1083. *Bottom:* Sequence of purified mutant allele, exon 4, family 5 showing 1235-1236 TA→AAG.

mechanistic boundaries. Phenotypic variability has previously been described for *PITX2* mutations.⁷⁻⁹ The high degree of intrafamilial variability is consistent with the observation that dominant *PITX2* mutations usually result in haploinsufficiency.

In several patients, significant differences were noted in the phenotype between the two eyes. In one case, (family 2, individual 2.2) this was the result of right-side, early-onset glaucoma. In two patients unilateral Peters' anomaly was diagnosed. Asymmetry or unilaterality is well recognized among ocular developmental disorders including congenital glaucoma and Peters' anomaly. It is of interest that recent observations suggest that *PITX2* has been shown to associate with genes

involved in lateralization and is likely to be one of the genes expressed late in the lateralization cascade.¹²⁻²² It is possible that the asymmetry observed in these patients reflects differences between the two sides in ocular development.

Before this, eight mutations had been reported in the *PITX2* gene.⁷⁻⁹ Five were missense mutations within the homeodomain (three in families with classic Rieger syndrome and two with iridogoniodysgenesis syndrome), two splicing mutations and one introducing a premature termination. By contrast, of the eight mutations described in our study, only two (families 6 and 8) were missense mutations within the homeodomain. The first, in family 2, converts a lysine to a glutamine at position 50 of the homeodomain—amino acid 9 of the recognition α -helix of the DNA-binding site. This lysine residue characterizes the homeodomains of the bicoid-related proteins of *Caenorhabditis elegans*, *Drosophila*, and murine *Otx1* and *Otx2*.²³ Furthermore, experiments on *Xotx2*, a related *Xenopus* homeobox gene have shown that mutation of this lysine residue to glutamine at the same site within its homeobox domain abolishes the developmental effects of the mRNA.²⁴ The second homeobox mutation (C851T in family 8), converts the arginine at codon 90, which lies two residues away, to a cysteine residue.

Of the six remaining mutations described, two were splicing mutations within introns 2 and 3. Finally, there were four nonsense mutations within exon 4 that result in premature termination with the loss of the C-terminal domain, which shows high conservation between *PITX2* and *PITX3*. Our results suggest that mutations would result in functional haploinsufficiency, which is consistent with others' observations.²⁵ The sites of the mutations within the gene are shown in Figure 4.

All eight families described had extraocular manifestations, including abnormal umbilical development with resultant protrusion of the skin, and abnormal dental development. These phenotypic features are strong clinical indicators of a *PITX2* mutation in patients with anterior segment dysgenesis, in that they have been noted in at least one member of all families so far described. Additional extraocular manifestations observed included skeletal (cleft palate, pectus deformity), gastrointestinal (imperforate-anterior placed anus, Meckel's diverticulum), and digital (finger pulp deficiency) abnormalities.

Among the patients screened, 11 (58%) of 19 with classic Rieger syndrome were not found to carry such mutations. The techniques of SSCP and heteroduplex analyses are not 100%

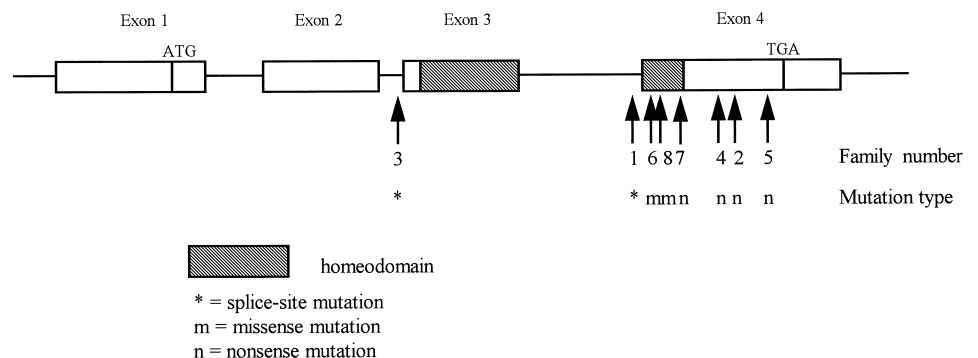


FIGURE 4. *PITX2* gene showing position of mutations described in this study.

* = splice-site mutation
 m = missense mutation
 n = nonsense mutation

sensitive and in particular do not detect whole exon or gene deletions. Nevertheless, this suggests that there is heterogeneity among patients with classic Rieger syndrome.

For families with Rieger syndrome, the major issue of concern is the visual outcome. This was generally better than for patients with *PAX6*-related phenotypes reflecting the absence of severe foveal hypoplasia, which was commented on in only one patient (family 1, patient 2.1) who had a best corrected visual acuity of 6/18. The major risk factors for adverse visual outcome that we have identified among patients with *PITX2* mutations include corneal opacification and early-onset glaucoma. Early-onset developmental glaucoma was diagnosed in 5 of 9 of the patients examined, although of the 10 patients, only 1, now an adult, had blindness caused by end-stage glaucoma. Patients and at-risk relatives should have life-long screening for glaucoma. Early-onset glaucoma is generally resistant to medical treatment, but advances in the efficacy of surgical intervention mean that the prognosis for patients with *PITX2* mutations is relatively optimistic.

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