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, iridogoniodygenesis, 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’ anomaly. Mutations include premature terminations and splice-site and homeobox mutations, confirming that haploinsufficiency is 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 opac 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 iridogoniodygenesis 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’ anomaly, 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. Probands 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
mutations were found in 8 (11%) of 76 patients with anterior segment dysgenesis phenotypes including Peters’ anomaly, Axenfeld 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

**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, Axenfeld 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

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

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

<table>
<thead>
<tr>
<th>Family</th>
<th>Patient</th>
<th>Anterior Segment Phenotype</th>
<th>Glaucoma</th>
<th>Dental Abnormalities</th>
<th>Umbilical Abnormalities</th>
<th>Additional Findings</th>
<th>Visual Acuity</th>
<th>Mutation</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>1.1</td>
<td>NK</td>
<td>+</td>
<td>+</td>
<td>NK</td>
<td>Mild left foveal hypoplasia</td>
<td>NK</td>
<td>Not tested</td>
</tr>
<tr>
<td>1</td>
<td>2.1</td>
<td>L. Rieger anomaly, anterior polar cataract, R. Peters anomaly</td>
<td>–</td>
<td>+</td>
<td>NK</td>
<td>L 6/18; R LP</td>
<td>L 6/6; 2 (–); A→T</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.2</td>
<td>L. and R. Iris hypoplasia, PAS</td>
<td>–</td>
<td>+</td>
<td>NK</td>
<td>L 6/9; R 6/12</td>
<td>C ins 1083</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.1</td>
<td>L. Peters anomaly, R. Iris hypoplasia, Axenfeld anomaly</td>
<td>–</td>
<td>+</td>
<td>NK</td>
<td>L CF; R 6/9</td>
<td>C ins 1083</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.2</td>
<td>L. and R. Rieger anomaly</td>
<td>–L, +R</td>
<td>–</td>
<td>NK</td>
<td>L 6/9; R NLP</td>
<td>C ins 1083</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.2</td>
<td>L. and R. Severe iris hypoplasia simulating aniridia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>L 6/12; R 6/9</td>
<td>Ivs 2 (–1), G→C</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.1</td>
<td>L. and R. Rieger anomaly</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>L 6/9; R 6/24</td>
<td>Ivs 2 (–1), G→C</td>
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<tr>
<td>4</td>
<td>1.1</td>
<td>L. and R. Rieger anomaly</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>L NLP; R CF</td>
<td>Good</td>
<td>A del 939</td>
</tr>
<tr>
<td>4</td>
<td>2.1</td>
<td>L. and R. Mild iris hypoplasia, PAS</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>L 6/5; R 6/9</td>
<td>1251→1256, TA→AAG</td>
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<tr>
<td>5</td>
<td>1</td>
<td>L. and R. Rieger anomaly</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>L 6/5; R 6/9-2</td>
<td>A 845 T</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>L. and R. Rieger anomaly</td>
<td>NK</td>
<td>+</td>
<td>+</td>
<td>NK</td>
<td>Blind</td>
<td>AA del 808-869</td>
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<tr>
<td>7</td>
<td>1.2</td>
<td>NK</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>L 6/6; R 6/12</td>
<td>8451 T</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2.1</td>
<td>L. and R. Rieger anomaly, PAS</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>L 6/6; R 6/12</td>
<td>8451 T</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2.2</td>
<td>NK</td>
<td>NK</td>
<td>+</td>
<td>+</td>
<td>NK</td>
<td>Good</td>
<td>Not tested</td>
</tr>
<tr>
<td>8</td>
<td>1.1</td>
<td>L. and R. Rieger anomaly</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>Clef palate, learning difficulties</td>
<td>NK</td>
<td>Not tested</td>
</tr>
<tr>
<td>8</td>
<td>2.1</td>
<td>NK</td>
<td>+</td>
<td>+</td>
<td>NK</td>
<td>Clef uvula, edentulous</td>
<td>NK</td>
<td>Not tested</td>
</tr>
</tbody>
</table>

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.

**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 inferiorty. (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.
mechanistic boundaries. Phenotypic variability has previously been described for PITX2 mutations.\textsuperscript{7–9} The high degree of intrafamilial variability is consistent with the observation that dominant PITX2 mutations usually result in haploinsufficiency.\textsuperscript{9} 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.\textsuperscript{9} 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.\textsuperscript{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.\textsuperscript{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 \textit{Caenorhabditis elegans}, \textit{Drosophila}, and murine \textit{Otx1} and \textit{Otx2}.\textsuperscript{23,24} Furthermore, experiments on \textit{Xotx2}, a related \textit{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.\textsuperscript{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.\textsuperscript{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%...
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 lifelong 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.

References