Genetics and Phenotypes of RPE65 Mutations in Inherited Retinal Degeneration

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PURPOSE. To characterize the spectrum of RPE65 mutations present in 453 patients with retinal dystrophy with an interest in understanding the range of functional deficits attributable to sequence variants in this gene.

METHODS. The 14 exons of RPE65 were amplified by polymerase chain reaction (PCR) from patients' DNA and analyzed for sequence changes by single-strand conformation polymorphism (SSCP) and direct sequencing. Haplotype analysis was performed using RPE65 intragenic polymorphisms. Patients were examined clinically and with visual function tests.

RESULTS. Twenty-one different disease-associated DNA sequence changes predicting missense or nonsense point mutations, insertions, deletions, and splice site defects in RPE65 were identified in 20 patients in homozygous or compound heterozygous form. In one patient, paternal uniparental isodisomy (UPD) of chromosome 1 resulted in homozygosity for a probable functional null allele. Eight of the disease-associated mutations (Y79H, E95Q, E102X, D167Y, 669delCA, IVS7+4a→g, G436V, and G528V) and one mutation likely to be associated with disease (IVS6+5g→a) have not been reported previously. The most commonly occurring sequence variant identified in the patients studied was the IVS1+5g→a mutation, accounting for 9 of 40 (22.5%) total disease alleles. This splice site mutation, as well as R91W, the most common missense mutation, exists on at least two different genetic backgrounds. The phenotype resulting from RPE65 mutations appears to be relatively uniform and independent of mutation class, suggesting that most missense mutations (15 of 40 disease alleles [37.5%]) result in loss of function. At young ages, this group of patients has somewhat better subjective visual capacity than is typically associated with Leber congenital amaurosis (LCA) type I, with a number of patients retaining some useful visual function beyond the second decade of life.

CONCLUSIONS. RPE65 mutations account for a significant percentage (11.4%) of disease alleles in patients with early-onset retinal degeneration. The identification and characterization of patients with RPE65 mutations is likely to represent an important resource for future trials of rational therapies for retinal degeneration. (Invest Ophthalmol Vis Sci. 2000;41:4293–4299)

The retinal pigment epithelium (RPE) performs a number of functions critical for visual processing, metabolism, and survival of the photoreceptor cells of the retina.1 Mutations in genes expressed in the RPE have recently emerged as an important cause of inherited retinal degenera-

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Swedish Briard dogs that carry a functional null allele of RPE65. These findings indicate that loss of RPE65 function results in a block in retinoid processing after esterification of vitamin A to membrane lipids, however, the mechanism by which RPE65 participates in retinoid isomerase activity of the RPE remains to be elucidated.

Inherited defects in vitamin A metabolism and other RPE-specific functions are likely to have a unique significance for research into the causes and treatment of retinal degeneration, since disorders affecting the RPE are, in principle, more accessible to therapeutic intervention than disorders directly affecting the proteins of the photoreceptor cells. Patients with RPE-specific defects may therefore be candidates for targeted therapies likely to become available in the near future. A specific defects may therefore be candidates for targeted therapies likely to become available in the near future.

We now report the results of RPE65 mutation analysis in a large collection of patients with retinal dystrophy from the United States and Europe. Our findings include the identification of a number of novel mutations. Our data are presented in the context of other known RPE65 mutations, including analysis of mutation class, prevalence, and associated phenotype.

RESULTS

Mutation Screening

To determine the relative contribution of RPE65 mutations to the causes of inherited retinal degenerations, we screened a group of 453 unrelated patients with various forms of retinal dystrophy using a combination of SSCP and direct DNA sequencing. Our analysis resulted in the identification of 21 different disease-associated DNA sequence variants in 20 patients that predict missense or nonsense point mutations, insertion, deletion, and splice site defects in RPE65 (Table 1, upper). These sequence variants were present in homozygous (homo) form in 7 patients and in compound (cmpd) heterozygous form in 13 patients. Disease relevance was suggested by segregation analysis (data not shown), with two notable observations. In one case (patient arRP850), two different sequence variants (1114delA and T457N) were detected on the same allele. Because 1114delA predicts a functional null mutation, the pathogenic potential of the T457N missense mutation cannot be determined. In a second case (patient 1725), segregation data were not compatible with Mendelian inheritance, in that the homozygous IVS1+5g→a mutation present in the patient was found to be carried only by his father. Analysis of three DNA polymorphisms in RPE65 and 29 genetic markers spread out along both arms of chromosome 1 showed the patient to be homozygous for the paternal allele in all cases, with no inconsistencies seen for any other chromosome tested (data not shown). These findings indicate that the patient is homozygous for the IVS1+5g→a mutation due to uniparental isodisomy (UPD) of chromosome 1 (Thompson D, Gal A, unpublished observations, June 2000).

In eight additional patients, six DNA sequence variants were detected on only one RPE65 allele (Table 1, lower). The disease relevance of these three DNA sequence variants (R85H, K294T, and N321K) is uncertain, because in two cases (R85H and N321K), the patients were heterozygous, although they were the children of consanguineous marriages. In addition, the case of N321K, this sequence change was detected (on one allele) in screens of 50 control individuals from the general population. This variant, however, has been detected in an unrelated patient in apparently compound heterozygous form. In the third case (K294T), in one of three families, only one of two affected siblings carried this sequence change. In contrast, disease relevance seems likely for the other three sequence variants identified in heterozygous form (IVS1+5g→a, A132T, and IVS6+5g→a), because these are either found in unrelated patients in homozygous form (this work and Reference 4) and/or predict functional null alleles. In the latter three heterozygous patients, mutation screening of an additional 650 bp of sequence from the RPE65 proximal promoter region, as well as the sequence from −2361 to −2599 containing potential Oct-1 and E47/Th1 sites, did not

Genotype Analysis

For analysis of individual mutation-associated haplotypes the microsatellite-type polymorphism located 2.8 kb upstream of the RPE65 transcription start site (locus D1S2803) was used.

METHODS

Subjects

RPE65 mutation screening was performed in patients with retinal dystrophy representing diverse forms of the disease. Informed consent was obtained from the patients, according to study protocols approved by university internal review boards for human subject studies. Our research followed the tenets of the Declaration of Helsinki.

Patient Evaluation

Ophthalmic examinations of patients treated in our clinics included slit lamp biomicroscopy; assessment of visual acuity, color perception, and visual fields; electroretinograms (ERGs); and ascertainment of family history. For other patients, clinical descriptions were provided by local ophthalmologists.

Mutation Screening

DNA was prepared from whole blood using standard methods. After polymerase chain reaction (PCR) amplification of individual or groups of exons, single-strand conformation polymorphism (SSCP) analysis was used to screen for DNA sequence changes in and near gene coding regions using oligonucleotide primers and conditions published previously. Direct DNA sequence analysis using chain terminator cycle sequencing technology (Amersham Pharmacia Biotech, Piscataway, NJ) with the same primer pairs used for PCR amplification was used to confirm suspected DNA sequence changes, as well as for primary screening in approximately one third of cases.

Sequence Analysis

Secondary structure predictions were made using the predictive algorithms of Chou and Fasman and Garnier et al. Potential posttranslational modification sites were identified using Prosearch to scan the Prosite database. Coefficients of splice site efficiency were calculated according to Shapiro and Senapathy.

Genotype Analysis

For analysis of individual mutation-associated haplotypes the microsatellite-type polymorphism located 2.8 kb upstream of the RPE65 transcription start site (locus D1S2803) was used.
result in the identification of a second disease-associated sequence variant.

**Missense Mutations**

The disease-associated missense mutations detected in our studies predict amino acid substitutions resulting in changes in charge (R91W, R91Q, E95Q, and D167Y), polarity (Y79H and Y368H), translation initiation (M1T), and (potentially) structural changes also did not disrupt predicted posttranslational modification sites, including consensus sequences for N-linked glycosylation, protein kinase C phosphorylation, casein kinase II phosphorylation, tyrosine kinase phosphorylation, and N-linked myristoylation. It should be noted, however, that the functional significance of these sites has not been established, and there is evidence that the mature protein contains neither O- nor N-linked glycans. Italic, sequence variants reported in this study also identified in patients in other studies.4,6,8 Nuclear types positions are numbered from the transcription initiation site located 54 bp upstream of the initiation codon.

**Table 1. Summary of RPE65 Sequence Variants Detected in Patients with Retinal Dystrophy**

<table>
<thead>
<tr>
<th>Exon/Intron</th>
<th>Mutation</th>
<th>Predicted Effect</th>
<th>Restriction Site</th>
<th>Patient</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 1</td>
<td>56T→C</td>
<td>M1T</td>
<td>+Mae II</td>
<td>arRP192</td>
<td>Cmpd</td>
</tr>
<tr>
<td>Intron 1</td>
<td>IVS1+5g→a</td>
<td>Inactive splice site</td>
<td>+Ssp 1</td>
<td>arRP181</td>
<td>Homo</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>arRP341</td>
<td>Cmpd</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>arRP69?</td>
<td>Cmpd</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>arRP713</td>
<td>Cmpd</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>arRP850</td>
<td>Cmpd</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>LCA821</td>
<td>Cmpd</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1725</td>
<td>Homo/UPD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2711</td>
<td>Cmpd</td>
</tr>
<tr>
<td>Exon 2</td>
<td>144ins T</td>
<td>Frame shift</td>
<td>+Mae 1</td>
<td>arRP341</td>
<td>Cmpd</td>
</tr>
<tr>
<td>Exon 3</td>
<td>289T→C</td>
<td>Y79H</td>
<td>No enzyme</td>
<td>arRP76</td>
<td>Cmpd</td>
</tr>
<tr>
<td>Exon 4</td>
<td>325C→T</td>
<td>R91W</td>
<td>-Rsa 1</td>
<td>arRP192</td>
<td>Cmpd</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>LCA820</td>
<td>Homo</td>
</tr>
<tr>
<td>Exon 5</td>
<td>326G→A</td>
<td>R91Q</td>
<td>+Sun 1</td>
<td>1024</td>
<td>Cmpd</td>
</tr>
<tr>
<td>Exon 6</td>
<td>337G→C</td>
<td>E95Q</td>
<td>+Hind I</td>
<td>arRP76</td>
<td>Cmpd</td>
</tr>
<tr>
<td>Exon 4</td>
<td>344del 20</td>
<td>Frame shift</td>
<td>+Kpn I</td>
<td>1348</td>
<td>Homo</td>
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<tr>
<td>Exon 4</td>
<td>358G→T</td>
<td>E102X</td>
<td>-Apo 1</td>
<td>arRP489</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>arRP69?</td>
<td>Cmpd</td>
</tr>
<tr>
<td>Exon 5</td>
<td>424C→T</td>
<td>R124X</td>
<td>-Taq 1</td>
<td>arRP114</td>
<td>Cmpd</td>
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<tr>
<td>Exon 6</td>
<td>533G→T</td>
<td>D167Y</td>
<td>-Mbo 1</td>
<td>arRP188</td>
<td>Cmpd</td>
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<tr>
<td>Exon 6</td>
<td>689del CA</td>
<td>Frame shift</td>
<td>+Mae 1</td>
<td>arRP192</td>
<td>Cmpd</td>
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<td>Exon 7</td>
<td>754C→T</td>
<td>R234X</td>
<td>+Allu N 1</td>
<td>1024</td>
<td>Cmpd</td>
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<tr>
<td>Intron 7</td>
<td>IVS7+4a→g</td>
<td>Inactive splice site</td>
<td>+Mae 1</td>
<td>1024</td>
<td>Cmpd</td>
</tr>
<tr>
<td>Exon 8</td>
<td>831del 8</td>
<td>Frame shift</td>
<td>+Hind I</td>
<td>arRP188</td>
<td>Cmpd</td>
</tr>
<tr>
<td>Exon 8</td>
<td>IVS8+1g→t</td>
<td>Inactive splice site</td>
<td>+Mae 1</td>
<td>PMK18/1</td>
<td>Homo</td>
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<tr>
<td>Exon 10</td>
<td>1114delA</td>
<td>Frameshift</td>
<td>No enzyme</td>
<td>arRP850</td>
<td>Cmpd</td>
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<tr>
<td>Exon 10</td>
<td>1144C→A</td>
<td>P363T</td>
<td>-BspM 1</td>
<td>PMK30/265</td>
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<tr>
<td>Exon 10</td>
<td>1156T→C</td>
<td>Y368H</td>
<td>+Nla III</td>
<td>arRP1</td>
<td>Cmpd</td>
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<tr>
<td>Exon 12</td>
<td>1361G→T</td>
<td>G436V</td>
<td>+Rsa 1</td>
<td>LCA821</td>
<td>Cmpd</td>
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<tr>
<td>Exon 14</td>
<td>1637G→T</td>
<td>G528V</td>
<td>+Rsa 1</td>
<td>LCA816</td>
<td>Cmpd</td>
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<tr>
<td>Intron 1</td>
<td>IVS1+5g→a</td>
<td>Inactive splice site</td>
<td>+Mae 1</td>
<td>LCA826</td>
<td>Hetero</td>
</tr>
<tr>
<td>Exon 4</td>
<td>308G→A</td>
<td>R85H</td>
<td>+Mae 1</td>
<td>LCA826</td>
<td>Hetero</td>
</tr>
<tr>
<td>Exon 5</td>
<td>448G→A</td>
<td>A132T</td>
<td>No enzyme</td>
<td>arRP476</td>
<td>Homo</td>
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<tr>
<td>Intron 6</td>
<td>IVS6+5g→a</td>
<td>Inactive splice site</td>
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<td>208</td>
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<tr>
<td></td>
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<td></td>
<td>858</td>
<td>Hetero</td>
</tr>
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<td>1369</td>
<td>Hetero</td>
</tr>
<tr>
<td>Exon 9</td>
<td>953A→C</td>
<td>K294T</td>
<td>No enzyme</td>
<td>PMK29/1</td>
<td>Hetero</td>
</tr>
<tr>
<td>Exon 13</td>
<td>1017T→G</td>
<td>N321K</td>
<td>No enzyme</td>
<td>arRP850</td>
<td>Cmpd</td>
</tr>
<tr>
<td>Exon 13</td>
<td>1424C→A</td>
<td>T457N</td>
<td>+Tsp509 1</td>
<td>PMK29/1</td>
<td>Hetero</td>
</tr>
</tbody>
</table>

Homo, homozgyous; Hetero, heterozygous; Cmpd, compound heterozygous. Key to fonts: Bold, novel sequence variants reported in this study; Bold italics, novel sequence variants reported in our previous studies; Regular, sequence variants reported in this study also identified in patients in other studies; Italics, sequence variants reported in our previous study also identified in patients in other studies.4,6,8 Nucleotide positions are numbered from the transcription initiation site located 54 bp upstream of the initiation codon.
IVS1+5g→a  R91W
1  2  3  4  5  6

1-4  1-1  2-3  1-3  3-3  1-2

**Figure 1.** Genotype analysis of six patients with retinal dystrophy with RPE65 mutations IVS1+5g→a or R91W using D15S2803. Alleles of the RPE65 intragenic microsatellite polymorphism D15S2803 were determined for patients with IVS1+5g→a mutations: arRP713 (cmpd), arRP181 (homo), and arRP341 (cmpd) (lanes 1, 2, and 3); and for patients with R91W mutations: arRP192 (cmpd), LCA820 (homo), and arRPL (cmpd; lanes 4, 5, and 6). The data indicate that both IVS1+5g→a and R91W arose independently on at least two different alleles.

not shown). Thus, the effect of missense mutations is unlikely to be at the level of the transcript. Disease-associated missense mutations in this population (15 of 40 disease alleles [37.5%]) occurred with slightly lower frequency than mutations predicting functional null alleles.

**Null Mutations**

In our studies, more than half of the disease-associated DNA sequence variants identified (11/20) predicted functional null alleles resulting from nonsense mutations (E102X, R124X, and R234X), a 1-bp insertion (144insT), small deletions (344del20, 669delCA, 831del8, and 1114 delA), and splice site mutations (IVS1+5g→a, IVS7+4a→g, and IVS8 +1g→t). The mutations are not clustered and are likely to result in the production of truncated protein, in some cases containing unrelated amino acid residues, or more likely, in complete absence of the protein due to greatly reduced mRNA/protein stability.

**RPE65 Mutation Prevalence**

In our total population of 455 patients screened, 339 were from an unselected collection of patients with retinal dystrophy, and 114 were included on the basis of a clinical diagnosis of LCA or early-onset retinal dystrophy. Of the latter 114 patients, 13 were found to have mutations in both RPE65 alleles (11.4%). Our data further show that RPE65 mutations accounted for 2.1% (7/339) of patients with autosomal recessive retinal dystrophy. The IVS1+5g→a mutation was the most common of all sequence variants identified, accounting for 9 of 40 total disease alleles (22.5%). Haplotype analysis indicated that the IVS1+5g→a allele occurred on at least two genetic backgrounds (Fig. 1, lanes 1, 2, and 3). Substitutions at arginine-91 were also common, with mutations at this position occurring in four families. Analysis of these families indicated that the R91W allele also arose independently on at least two genetic backgrounds (Fig. 1, lanes 4, 5, and 6). Among single nucleotide changes, transitions (13/23) occurred at a higher frequency than transversions (10/23). Eight of the disease-associated mutations identified in the present study (Y79H, E95Q, E102X, D167Y, 669delCA, IVS7+4a→g, G456V, and G528V) and one mutation likely to be associated with disease (IVS6+5g→a) have not been reported previously.

**Patient Phenotypes**

The patients included for screening in our study were affected by retinal degeneration associated with a range of disease presentations, with many instances of severe, early-onset forms of disease represented. The phenotype of patients carrying RPE65 mutations, however, appeared to be more uniform than that of the screening population as a whole. A summary of relevant data for patients with both disease alleles identified is shown in Table 2. In most patients with RPE65 mutations, disease was diagnosed in infancy, with visual impairment frequently associated with nystagmus, night blindness, and a tendency to fixate on light. Photophobia was not observed in this group. Constricted visual fields were documented at young ages when measured. In most cases, the retina appeared pale without significant pigment accumulation and with RPE atrophy in the periphery. The data available for very young patients indicate that rod ERGs are undetectable, and cone ERGs is severely diminished at the earliest ages measured (approximately 1 year old), with cone ERGs becoming undetectable by 5 to 7 years of age. Despite such poor ERG indicators, the visual performance of several patients in bright light was sufficient to permit attendance at regular school during the elementary years. At older ages, often during the secondary school years, visual acuity was greatly reduced. However, a number of patients retained residual islands of central or peripheral vision into their third decade, with only one report of no light perception in a patient 25 years old. In the group of 20 patients in whom disease-associated mutations in both RPE65 alleles were identified, 10 (50%) patients had two apparent null alleles, 5 (25%) patients had two missense mutations, and 5 (25%) patients had both an apparent null allele and a missense mutation. However, no obvious difference in visual performance appeared to exist among patients affected with different categories of mutations. For example, patient arRP341 carried two apparent null alleles (IVS1+5g→a, 144insT), patient LCA820 carried a homozygous missense mutation (R91W), and patient 2711 carried a missense mutation and an apparent null allele in compound heterozygous form (Y368H, IVS1+5g→a). At young ages, the clinical descriptions and best visual acuities of all three patients (20/100–20/200) were virtually indistinguishable.

**Discussion**

As a result of screening a large and diverse collection of individuals with various presentations of retinal dystrophy, we found that RPE65 mutations are a common cause of congenital or early-onset retinal degeneration, responsible for the disease in 11.4% of patients with mutations present in homozygous or compound heterozygous form. This prevalence is approximately equal to that reported for RPE65 mutations in patients with LCA in whom both mutations were identified in two earlier studies (7/45, 15.6%; 5/54, 9.3%) and is greater than that reported in a recent study (7/176, 4%). UPD, the situation in which an individual inherits two copies of a specific chromosome from one parent and no copy from the other, was also identified in our studies as the cause of retinal dystrophy in one patient as a result of reduction to homozygosity for RPE65. The disease-associated mutations identified by us,
along with other published \textit{RPE65} mutations, are shown on the schematic of the gene structure in Figure 2. The mutations identified in our studies account for approximately half of all mutations currently known, and are representative of the group as a whole in terms of mutation type, location, and associated phenotype.

The autosomal recessive nature of the disease resulting from \textit{RPE65} mutations predicts that missense mutations result in loss or considerable reduction of protein function. For two of the missense mutations identified in our studies, R91W and P363T, the connection to disease pathogenesis is very likely, because these sequence variants segregate with the disease phenotype in patients in homozygous form. This was also the case for the mutations M1T and A132T identified in homozygous form by others. Most of the other missense mutations identified in our studies (Y79H, R91Q, E95Q, D167Y, P363T, Y368H, G436V, and G528V) are likely to be pathogenic based on genetic evidence. In contrast, there was no strong evidence linking four of the identified missense mutations (R85H, K294T, N321K, and T457N) to the disease state. These se-

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Patient & Mutation & Age at Onset and Diagnosis & Visual Acuity, Visual Fields, Refraction & ERG Data & Comments and Recently Ascertained Visual Function \\
\hline
arRPL & R91W, Y368H & 7 mo, nystagmus, fixation on light & 7 y, VA 20/100; VF V4+; II4+, II4− & 7 y, rod and cone ERGs residual; 7 y, ERGs nonrecordable & 7 y, current age$^a$ \\
\hline
arRP76 & Y79H, E95Q & 3 y, nightblind; 20 y, diagnosis RP & No data & No data & 40 y, able to read; 58 y, small islands of residual vision \\
\hline
arRP114 & R124X, R234X & Birth, nightblind; 5 y, diagnosis RP & No data & No data & 17 y, able to read; 42 y, LPO \\
\hline
arRP181 & IVS1+5g→a, IVS1+5g→a & Young child, nightblind & No data & No data & Attended regular school, 10 y, RP diagnosis; 14 y, able to read; 18 y, legally blind \\
\hline
arRP188 & D167Y, 831del8 & Birth, nightblind & No data & No data & 19 y, unable to walk alone; 25 y, no LP$^†$ \\
\hline
arRP192 & M1T, R91W & 2 y, nightblind & 6 y, central VA acceptable in bright light & No data & 18 y, poor VFs; 38 y, able to read; 48 y, LPO \\
\hline
arRP341 & IVS1+5g→a, IV4ins T & 4 mo, nystagmus, fixation on light & 6 y, VA 20/200, RE and 20/100 LE; VF III4+, III4− hyperopic & 1 y, rod absent, cone abnormal ERG; 6 y, ERGs nonrecordable & 8 y, VF and monocular VAs unchanged$^b$ \\
\hline
arRP697 & IVS1+5g→a, E102X & 2 y, nystagmus, nightblind; 6 y, onset of VA loss & Best VA 20/200, myopic & 18 y, ERG nonrecordable & 18 y, legally blind; 31 y, unable to read; 57 y, CF, VFs unrecordable \\
\hline
arRP713 & IVS1+5g→a, 669del1CA & 3 y, nystagmus, nightblind, poor VA & 16 y, VA 20/200, VF V4 20°, III4−, hyperopic & 29 y, ERG nonrecordable & 30 y, VA 20/600; 35 y, LPO, VFs nonrecordable \\
\hline
arRP849 & E102X, E102X & Infant, nystagmus, nightblind, poor VA & 6 y, VF ring scotomas & 6 y, rod ERG absent; 12 y, cone ERG absent & 16 y, VA 20/400 \\
\hline
arRP850 & IVS1+5g→a, T457N, 1114delA & 3 y, nystagmus & 4 y, VA 20/100; VF III4+, III4− hyperopic, astigmatism & 6 y, rod and cone ERGs nonrecordable & 6 y, current age$^a$ \\
\hline
LCA816 & 669del1CA, G528V & 6 mo, nystagmus, nightblind, poor VA & Best VA 20/200, myopic, astigmatism, 15 y, VF III4−, V4 10° & 15 y, ERG nonrecordable & Attended school for the blind, 30 y, no LP and 20/400, VF nonrecordable \\
\hline
LCA820 & R91W, R91W & 3 y, nystagmus, nightblind, poor VA & Best VA 20/200, plano 23 y, VF V4 8° & 23 y, ERG nonrecordable & 30 y, VA 20/100 and 1/40, VF V4 5° \\
\hline
LCA821 & IVS1+5g→a, G436V & Birth, nystagmus, nightblind, poor VA & Best VA 20/100, hyperopic, astigmatism & 19 y, ERG nonrecordable & 19 y, rapid deterioration; 21 y, VF V4 5°; 25 y, VA 20/200 and 1/40 \\
\hline
PMK18/1 & IVS8+1g→t, IVS8+1g→a & Young child & 9 y, VA 20/60 RE, CF LE & No data & 11 y, current age$^†$ \\
\hline
PMK30/265 & P563T, P563T & 3–7 y, nystagmus, nightblind & CF and hand movements & No data & Severe visual handicap between 5 and 12 y; Residual central and peripheral field islands \\
\hline
1024 & R91Q, IVS7+4a→g & Toddler, nystagmus, nightblind & 20 y, VA 20/400; refraction −200 & 20 y, rod ERG absent, cone ERG abnormal & Residual central and peripheral field islands \\
\hline
1348 & 34del20, 34del120 & Before 5 y, nystagmus & 7 y, VA 20/200; refraction +100 & 7 y, abnormal cone ERG & 16 y, entered school for blind using low vision aids \\
\hline
1723 & IVS1+5g→a, IVS1+5g→a & Before 5 y, nystagmus & 8 y, VA 20/200; refraction +400 & 35 y, rod and cone ERGs not detectable & 50 y, small residual islands of vision \\
\hline
2711 & IVS1+5g→a, Y368H & 5 y, nystagmus, nightblind & 5 y, VA 20/60–20/100; 10 y, VF 90° to V4e, cannot see V2e & 5 y, rod and cone ERGs not detectable & 30 y, VF peripheral residual islands only to IV4e, V4e \\
\hline
\end{tabular}
\end{table}

$^a$ For clinical description, see Lorenz et al.

$^†$ For clinical description, see Gu et al.
quence changes may therefore represent rare variants of the RPE65 gene which are not, in themselves, causal for the observed phenotype, but which could play a role, for example, in multifactorial retinal diseases with late onset.37 Indirect evidence that certain amino acid substitutions produce subtle changes in RPE65 activity has been obtained in recent studies showing association between an Rpe65 polymorphism and susceptibility to light damage in strains of inbred mice.28 As the characterization of the vitamin A cycle continues to develop on a molecular level, it should be possible to devise strategies to explore the possibility that defects in multiple genes may have an interactive effect in causing disease.

The mechanisms by which RPE65 mutations, in general, contribute to pathogenesis are not yet known and, in part, await elucidation of the specific role of the protein in RPE physiology. Because all known missense mutations affect highly conserved residues but do not appear to disrupt protein folding or posttranslational processing and do not cluster within the linear protein sequence, it may be that these mutations inactivate a functional domain or domains present in the folded protein structure. Such mutations may be predicted to interfere with protein–protein interactions, subcellular localization, ligand binding, or intrinsic enzymatic activity necessary for the synthesis of 11-cis retinal. Each of these hypotheses will be testable when assays of the specific function(s) of the RPE65 protein become available.

Our inability to detect a second RPE65 mutant allele in three patients identified as having one probable disease-associated mutation, a general finding also reported by other groups,6,8,21 may be due to the presence of large deletions or other rearrangements undetected by current screening methods. Alternatively, mutations may occur in other regions of the RPE65 gene not analyzed in our study, including promoter and intronic regions. Mutation screening of these sequences is not practical at this time, because the critical elements that regulate RPE65 promoter activity have not yet been identified,20 and no highly conserved intronic sequences beyond the splice site consensus sequences are known.29 Other possibilities include dominant effect of the mutation, digenic inheritance, or interference with protein–protein interactions, subcellular localization, ligand binding, or intrinsic enzymatic activity necessary for the synthesis of 11-cis retinal. Each of these hypotheses will be testable when assays of the specific function(s) of the RPE65 protein become available.

Such a correlation has also been proposed to exist for disease severity and mutations in the ATP-binding cassette transporter of rods (ABCR) gene in autosomal recessive Stargardt disease, fundus flavimaculatus, cone–rod dystrophy, and retinitis pigmentosa.32 However, our studies now show that a severe phenotype can result from a number of different combinations of RPE65 null and missense mutations. Together, these findings suggest the possibility that some missense mutations may result in true null alleles, whereas others may simply reduce the effectiveness of the protein product. Alternatively, or in addition, variability in disease severity may be determined by modifier genes that impact RPE65-related cell biology. Resolution of this issue also awaits the development of functional tests of mutant RPE65 protein.

The initial reports describing RPE65 mutations defined the associated phenotype as a childhood-onset, severe retinal dystrophy2 and as LCA.3 Subsequently, it has been proposed that patients with LCA who have RPE65 mutations can be distinguished from patients who have mutations in the photoreceptor-specific guanylate cyclase gene, RetGC1, on clinical grounds.5,33 We find that many RPE65 patients share a common phenotype characterized by poor but useful visual function in early life (measurable cone ERGs) that declines dramatically throughout the school age years. In addition, a number of these patients retain residual islands of peripheral vision, although considerably compromised, into the third decade of life. Thus, the phenotype resulting from RPE65 mutations appears relatively uniform, possibly because each mutation exerts its effect by producing similar deficits in RPE function. It seems likely that the use of various diagnostic designations for these patients, including LCA II, early-onset severe retinal dystrophy, autosomal recessive retinal dystrophy, and early severe retinitis pigmentosa, merely reflects usage preferences by individual ophthalmologists rather than actual phenotypic differences that define patient subtypes.

The phenotype and functional defects resulting from RPE65 mutations, as well as the existence of both mouse and canine models of the disease, makes this patient population attractive candidates for future therapeutic trials focused on manipulation of the vitamin A cycle. Patients with the RPE65 mutation who have onset of disease in infancy and who retain reasonable visual function that is lost only over the course of many years would seem to be ideal subjects for therapeutic intervention. Identifying these individuals at young ages will enhance therapeutic opportunities. Research from many laboratories over the next few years will determine which of the many approaches currently under study, including gene therapy, RPE transplantation, and retinoid and survival factor therapy, may have the greatest potential for success in this group of patients. Meanwhile, in anticipation of such trials, continued
characterization of this patient population, as well as the corresponding animal models, remain important goals for the immediate future.

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References