

A Comprehensive Mutation Analysis of *RP2* and *RPGR* in a North American Cohort of Families with X-Linked Retinitis Pigmentosa

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X-linked retinitis pigmentosa (XLRP) is a clinically and genetically heterogeneous degenerative disease of the retina. At least five loci have been mapped for XLRP; of these, *RP2* and *RP3* account for 10%–20% and 70%–90% of genetically identifiable disease, respectively. However, mutations in the respective genes, *RP2* and *RPGR*, were detected in only 10% and 20% of families with XLRP. Mutations in an alternatively spliced *RPGR* exon, *ORF15*, have recently been shown to account for 60% of XLRP in a European cohort of 47 families. We have performed, in a North American cohort of 234 families with RP, a comprehensive screen of the *RP2* and *RPGR* (including *ORF15*) genes and their 5' upstream regions. Of these families, 91 (39%) show definitive X-linked inheritance, an additional 88 (38%) reveal a pattern consistent with X-linked disease, and the remaining 55 (23%) are simplex male patients with RP who had an early onset and/or severe disease. In agreement with the previous studies, we show that mutations in the *RP2* gene and in the original 19 *RPGR* exons are detected in <10% and ~20% of XLRP probands, respectively. Our studies have revealed *RPGR-ORF15* mutations in an additional 30% of 91 well-documented families with X-linked recessive inheritance and in 22% of the total 234 probands analyzed. We suggest that mutations in an as-yet-uncharacterized *RPGR* exon(s), intronic changes, or another gene in the region might be responsible for the disease in the remainder of this North American cohort. We also discuss the implications of our studies for genetic diagnosis, genotype-phenotype correlations, and gene-based therapy.

Introduction

Retinitis pigmentosa (RP) is a clinically and genetically heterogeneous group of retinal degenerative diseases, characterized by night blindness, progressive restriction of the visual field, and pigmentary retinopathy. At least 28 different genetic loci have been mapped for autosomal dominant, autosomal recessive, and X-linked forms of RP (see RetNet Web site). The X-linked RP subtype (XLRP [MIM 268000]) is relatively severe, with an early age at onset and rapid progression, accounting for 10%–20% of families with RP (Bird 1975; Fishman 1978). XLRP is

genetically heterogeneous, with five mapped loci: *RP2* (MIM 312600) (Bhattacharya et al. 1984), *RP3* (MIM 312610) (Musarella et al. 1988), *RP6* (MIM312612) (Breuer et al. 2001), *RP23* (Hardcastle et al. 2000) and *RP24* (MIM 300155) (Gieser et al. 1998). On the basis of linkage analysis, *RP2* is predicted to account for 10%–20% and *RP3* for 70%–90% of XLRP (Musarella et al. 1990; Ott et al. 1990; Teague et al. 1994; Fujita et al. 1997). The *RP2* and *RPGR* (for *RP3*) genes for these two major loci have now been cloned (Meindl et al. 1996; Roepman et al. 1996; Schwahn et al. 1998).

The *RP2* gene is composed of five exons, encoding a ubiquitously expressed polypeptide of 350 amino acids (Schwahn et al. 1998). The N-terminus of the protein is homologous to cofactor C, which is involved in β -tubulin folding (Tian et al. 1996). A majority of the mutations in *RP2* are localized in the cofactor C homologous domain and are predicted to generate a truncated protein (Schwahn et al. 1998; Mears et al. 1999). Further sequence analysis and secondary structure predictions

Received February 19, 2002; accepted for publication March 21, 2002; electronically published April 30, 2002.

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show that residues 207–244 of the RP2 protein are homologous to a pig microtubule-associated protein, the γ -subunit of T-complex, and residues 250–317 show homology to NM23, a member of the nucleoside diphosphate kinase family (Miano et al. 2001). In transiently transfected cultured cells, the RP2 protein is localized to the plasma membrane, and mutations in the first five amino acids disrupt this localization (Chapple et al. 2000; Schwahn et al. 2001). These studies suggest that RP2 is a membrane- and/or tubulin-associated chaperone or signaling protein.

RPGR, the RP3 gene, spans 70 kb and was originally reported to include 19 exons encoding a protein of 815 amino acids. The N-terminal region of RPGR contains tandem repeats similar to RCC1 (called RCC1-like domain or RLD), which is a guanine nucleotide exchange factor (GEF) for Ran-GTPase (Ohtsubo et al. 1987; Drivas et al. 1990). On the basis of RCC1 crystal structure (Renault et al. 1998), the RPGR protein is predicted to have a seven-blade β -propeller structure and to function as a GEF for a small GTP-binding protein. Since the original reports (Meindl et al. 1996; Roepman et al. 1996), several groups have identified new exons and multiple RPGR transcripts (at least 14) with extensive alternative splicing (Yan et al. 1998; Kirschner et al. 1999; Vervoort et al. 2000; Kirschner et al. 2001). One of these new exons is *ORF15*, which is contiguous with exon 15 (located in the original intron 15). It spans 1.6 kb, is highly repetitive, and encodes a putative glutamic acid- and glycine-rich domain. *ORF15* was also shown to be a mutational hot spot, with presumed mutations in 28 of 47 patients with XLRP (Vervoort et al. 2000). Inherent complexity generated by extensive alternative splicing has produced inconsistent results of RPGR intracellular localization and made the functional studies difficult. A mouse model generated by an in-frame deletion of *Rpgr* exons 4–6 showed a phenotype of slow cone and rod degeneration (Hong et al. 2000). Two proteins, PDE- δ and RPGR-interacting protein (RPGRIP), have been identified by the yeast two-hybrid method (Linari et al. 1999; Boylan and Wright 2000; Roepman et al. 2000b). The RPGR and RPGRIP proteins have been localized to the connecting cilia and rod outer segments (Hong et al. 2001; Roepman et al. 2000a).

Consistent with genetic studies, mutations in RP2 are observed in 7%–18% of XLRP (Hardcastle et al. 1999; Mears et al. 1999). However, the reported mutation screens have shown a remarkably low frequency of disease-causing sequence changes (11%–26%) in RPGR (Buraczynska et al. 1997; Sharon et al. 2000). RPGR-*ORF15* was recently reported to account for 60% of missing mutations in a European XLRP cohort (Vervoort et al. 2000). Recently, RPGR-*ORF15* mutations have also been described in families with X-linked cone degeneration (Mears et al. 2000; Demirci et al. 2002;

Yang et al. 2002). These previous investigations, however, undertook limited mutation screenings of relatively small patient cohorts. To determine the spectrum of mutations in a large XLRP cohort from North America, we performed a comprehensive analysis of the RP2 and RPGR genes, including all reported exons and their 5' upstream regions. The results demonstrate putative disease-causing mutations in 56 (62%) of 91 well-documented North American families with XLRP and in 100 (43%) of the total of 234 probands analyzed.

Subjects, Material, and Methods

Patients and Families

We have performed mutation analysis in a cohort of 234 putative XLRP families of predominantly (>95%) white ancestry from North America. Although many families are believed to have mixed ethnicity, we can be certain only of five Hispanic and two African American families in our cohort. Of the 234 families, 160 were referred by collaborating clinicians, and 74 were obtained by referral from the Foundation Fighting Blindness as part of the Retinitis Pigmentosa Research Center (RPRC). The diagnosis of XLRP in the clinician-referred families was made by ocular examination, visual field testing, and/or electroretinograms (ERGs), as well as by pedigree analysis (Jacobson et al. 1989; Sieving 1995). For individuals referred by the RPRC, the clinical information was less complete and the diagnosis was based on ophthalmic examination and pedigree data. Families with XLRP were subdivided into three categories that reflected the strength of the clinical documentation and the family history of disease (see table 5). Of the clinician-referred patients, 39% had a family history with at least two generations of affected males that were related through an unaffected or carrier female (total of 91; class A). An additional 38% were referred either by clinicians or through the RPRC and reported a family history of two or more affected male subjects, in a single or multiple generations (total of 88; class B). The remaining probands were the only affected males in the family with RP (simplex males), but, in most instances, were given the tentative diagnosis of XLRP on the basis of clinical presentation (e.g., early onset and severity of disease) (total of 55; class C).

Informed consent was obtained from patients and healthy subjects after the nature of the procedures had been fully explained. The research protocols were approved by the institutional review boards of the University of Michigan and all other collaborating institutions and were in accordance with the Declaration of Helsinki. Referring clinicians provided control DNA samples from unaffected individuals with no family his-

tory of retinal disease. DNA was extracted from lymphocytes by use of standard procedures.

Mutation Analysis

Of the 234 families, 49 were previously screened for *RPGR* (Buraczynska et al. 1997) and 63 for *RP2* (Mears et al. 1999). In the present study, we performed the mutation screening of 185 probands for *RPGR* and 171 for *RP2* by directly sequencing the *RPGR* (including *ORF15*) and *RP2* genes, including their 5' upstream regions. The new mutations identified in this study were combined with the previously reported mutations for a comprehensive cohort analysis. Eighteen primer sets for *RPGR*-promoter exon 19 (Meindl et al. 1996), two primer sets for *ORF15* (Vervoort et al. 2000), and five primer sets for *RP2* exons (Schwahn et al. 1998) were synthesized at the Biomedical Research Core Facility of the University of Michigan and/or by Invitrogen. The primer sets amplify each exon and at least 25 bp of intronic sequence on either end. The primer pairs for the 5' upstream regions of *RP2* and *RPGR* were designed from the genomic sequences (GenBank accession numbers NT_011572 [*RP2*] and AJ318463 [*RPGR*]) and are as follows: *RP2*-1F: 5'-TTG-CTCGAGAGGCTTTGATT-3'; *RP2*-1R: 5'-GACTTCA-GCTATCCGCGTTC-3'; *RPGR*-1F: 5'-GACTAGCTGCCACATGCTTAAAC-3'; and *RPGR*-1R: 5'-GGATGGGGGAGAACGGGAAA-3'. Amplified products were purified either with Qiaquick columns (Qiagen) or with the MultiScreen PCR system (Millipore). Products were sequenced by the University of Michigan DNA sequencing core using reagents (BigDye version 1) and automated sequencers (models 373, 377, and 3700) from Applied Biosystems, according to the manufacturer's protocols. Sequence variations were confirmed using the ThermoSequenase Cycle Sequencing Kit (Amersham Pharmacia Biotech). Sequences were aligned using Sequencher (Gene Codes). Mutations were confirmed by analyzing available family members. Sequence variations were deemed putative mutations if they were predicted to result in a truncated protein, had not been previously reported as polymorphisms (Sharon et al. 2000; Vervoort et al. 2000), or were not identified in a screen of 200 normal chromosomes.

Results

Mutations and Sequence Variations in *RP2*

Direct sequencing of the PCR products spanning all exons, exon-intron boundaries, and the 5' upstream region of the *RP2* gene revealed nine different mutations in 10 of the 171 patients examined; of these, eight are novel mutations (table 1 and fig. 1). These mutations cosegregated with the disease in all available family members.

Table 1

Mutations in the *RP2* Gene in Affected Hemizygotes with XLRP

Mutation ^a	Protein Change ^b	Exon	Patient(s)
Deletion:			
409-411del	Ile37del	2	1694
350-351del	Phe117fsTer155	2	471
Insertion:			
515insG	Ser172fsTer173	2	790
670insC	Arg225fsTer234	2	1358
Splice site:			
IVS1+3A→G		Int1	115
Nonsense:			
82C→G	Tyr27Ter	1	1645
Missense:			
200G→A	Cys67Tyr	2	1699
353G→A	Arg118His	2	1324 and 1737
565T→C	Leu188Pro	2	1063

^a Nucleotide positions are based on GenBank sequence (accession number NM_006915), with the first nucleotide of the initiation codon ATG designated as base 1.

^b Frameshift mutations are designated according to the following example: Phe117fsTer155 refers to a frameshift mutation, in which Phe117 is the first amino acid altered, with termination of the open reading frame at residue 155.

Frameshift, nonsense, and splice-site mutations.—Four frameshift mutations—small deletions or insertions—were detected in exon 2 in four patients. Tyr27Ter, a novel nonsense mutation, was found in one of the probands. IVS1+3A→G is a novel mutation and is predicted to affect the acceptor site of intron 1. The same nucleotide was altered in a previously reported mutation as a transversion (Sharon et al. 2000). Six of the seven mutations in this group are predicted to generate a truncated protein before or within the cofactor C homology domain. The seventh mutation has been previously reported and would result in the deletion of Ile137, a conserved residue in the cofactor C homology domain.

Missense mutations.—Three missense mutations, two of which are novel, were found in four of the probands. These missense mutations were not observed in >200 chromosomes from healthy individuals. The Cys67Tyr mutation is predicted to change a potential disulfide bond-forming conserved residue to a larger neutral amino acid in the cofactor C homology domain. Arg118His and Leu188Pro are mutations in residues that are conserved between *RP2* and the human cofactor C protein.

Sequence variations and polymorphisms.—One polymorphic change, 844C→T, was observed in two patients in the mutation screen (table 2). This sequence variation has been reported elsewhere (Sharon et al. 2000) and was detected in both affected and unaffected individuals.

Mutations and Sequence Variations in *RPGR*

Analysis of *RPGR* exons 1–19 revealed eighteen different mutations in 19 of the 185 independent XLRP

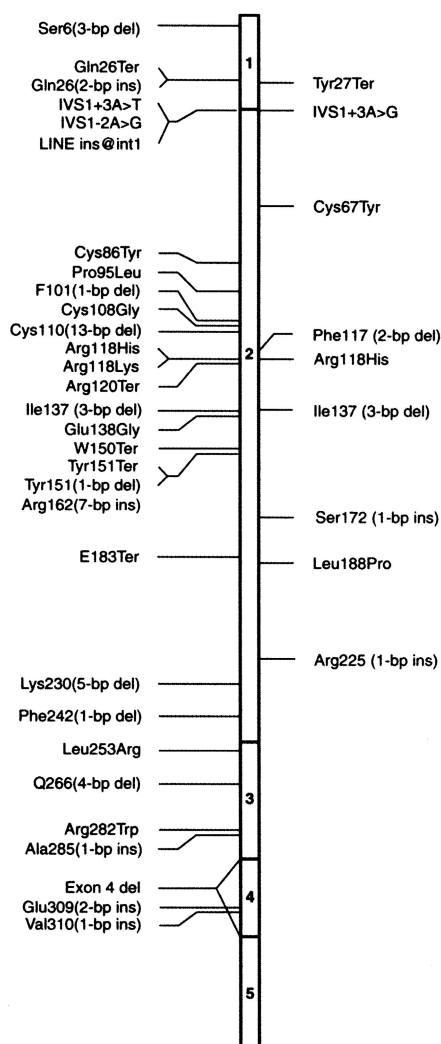


Figure 1 Location of all known mutations in *RP2*. The right side designates mutations identified in this screen; all previously reported mutations are shown on the left.

patients that were examined (table 3 and fig. 2). All mutations cosegregated with the disease in available family members.

Frameshift, nonsense, and splice-site mutations.—Of the 18 mutations, 11 (five frameshift, two splice-site, and four nonsense mutations) are predicted to result in a truncated protein. Two of the frameshift mutations have been reported previously, whereas four are novel. Nine of the mutations are predicted to truncate a part of the RLD. Two other mutations are predicted to truncate the protein after exon 11 or 14, thereby leaving the RLD intact but deleting the entire ORF15 region. An additional previously reported mutation is predicted to delete the Leu258 codon.

Two mutations, one of which is novel, were detected

in conserved residues at intron/exon splice sites. One of these is in the donor site of intron 4, and the other is in the donor site of intron 10. Four nonsense mutations, three of which are novel, were identified in four independent patients. Each of these is predicted to result in a truncation of the RPGR protein prior to exon 12 and delete a part of RLD.

Missense mutations.—In this mutation screen, we identified six missense mutations that were not detected in at least 200 normal chromosomes. One of these, Arg137Gly, has been previously reported, whereas the remaining five are novel. Gly173Arg affects a residue conserved between RPGR and RCC1, whereas Cys250Tyr, Gly266Arg, Glu285Gly, and Gly436Asp mutations alter residues in the RLD, supporting the importance of this domain in RPGR function.

Sequence variations and polymorphisms.—Twenty-

Table 2

Sequence Variations and Polymorphisms in the *RP2* and *RPGR* Genes

SEQUENCE CHANGE	LOCATION	% IN POPULATION	
		Patient	Unaffected ^a
<i>RP2</i> :			
844C→T ^b	Exon 3	1.6	ND
<i>RPGR</i> :			
8C→A	Promoter	2.2	2.5
IVS1-15A→G ^b	Intron 1	3.8	ND
212C→T	Exon 3	.5	ND
1146T→A	Exon 10	0	1.4
1223G→T ^b	Exon 10	13.0	4.9
IVS10+16A→G ^b	Intron 10	1.1	ND
1333G→A ^b	Exon 11	2.2	9.2
1350A→G ^b	Exon 11	1.1	6.3
1354A→G	Exon 11	0	.5
1426A→G	Exon 11	1.6	1.9
IVS12-101T→A ^b	Intron 12	2.7	ND
IVS12-100 1-bp ins ^b	Intron 12	2.2	ND
IVS12-97T→C ^b	Intron 12	1.6	ND
IVS12-93 2-bp ins ^b	Intron 12	13.5	ND
IVS13+11A→G ^b	Intron 13	1.6	ND
1635-1637del	Exon 14	1.1	ND
1657C→T	Exon 14	1.1	1.4
1746G→A	Exon 14	2.7	7.2
IVS16-137T→A	Intron 16	2.7	ND
IVS17+46C→T ^b	Intron 17	2.7	ND
IVS18+11T→C ^b	Intron 18	23.2	ND
<i>RPGR-ORF15</i> : ^c			
863ins, 21 bp		.8	ND
915-917del		.8	ND
965-967del		7.4	ND
1052-1072dup		1.7	ND
1221-1235del		.8	ND
1299-1310del		9.1	ND
1309-1320del		.8	ND

^a ND = not determined.

^b Previously identified polymorphisms (Sharon et al. 2000).

^c Previously identified polymorphisms (Vervoort et al. 2000).

Table 3

Mutations in the *RPGR* Gene in Affected Hemizygotes with XLRP

Mutation ^a	Protein Change ^b	Exon	Patient(s)
Deletion:			
541-542del	Phe162fsTer165	6	913
688-698del	Glu210fsTer214	7	310
830-832del	Leu258del	7	652
1431-1432del	Cys458fsTer461	11	774
1744-1745del	His562fsTer581	14	688
Insertion:			
1335ins 2bp	Ile407fsTer452	10	770 and 413
Splice site:			
IVS4+1 G→C		Int4	163
IVS10+2 T→C		Int10	1689
Nonsense:			
474G→T	Glu139Ter	5	333
551G→A	Trp164Ter	6	102
1293C→T	Arg412Ter	10	725
1404C→T	Arg449Ter	11	902
Missense:			
438A→G	Arg127Gly	5	1745
576G→C	Gly173Arg	6	1734
808G→A	Cys250Tyr	7	555
859G→A	Gly266Arg	8	1708
924A→G	Glu285Gly	8	1661
1366A→G	Gly436Asp	11	767

^a Nucleotide positions and nomenclature are based on the work of Meindl et al. (1996) and Buraczynska et al. (1997).

^b Frameshift mutations are designated according to the following example: Phe162fsTer165 refers to a frameshift mutation, in which Phe162 is the first amino acid altered, with termination of the open reading frame at residue 165.

one sequence variations, nine of which are novel, were identified in this study. Table 2 lists the variations and their frequency in our normal and patient cohort.

Mutations in RPGR-ORF15

The *RPGR* exon *ORF15* was analyzed for mutations by direct sequencing of PCR products with multiple internal primers. Our analysis revealed 21 different mutations in 41 of the 195 patients examined and includes 17 frameshift and four nonsense mutations (table 4 and fig. 3). All of the frameshift mutations alter the predicted protein sequence for at least 19 amino acids prior to termination. One of these is predicted to truncate the *RPGR* protein after 86 altered amino acids.

Discussion

This is the first comprehensive report of mutation screening in a large cohort affected with XLRP in which the *RP2* and *RPGR* (including *ORF15*) genes and their 5' upstream regions were directly sequenced in affected male probands. Our results establish a molecular definition of XLRP in a North American population and

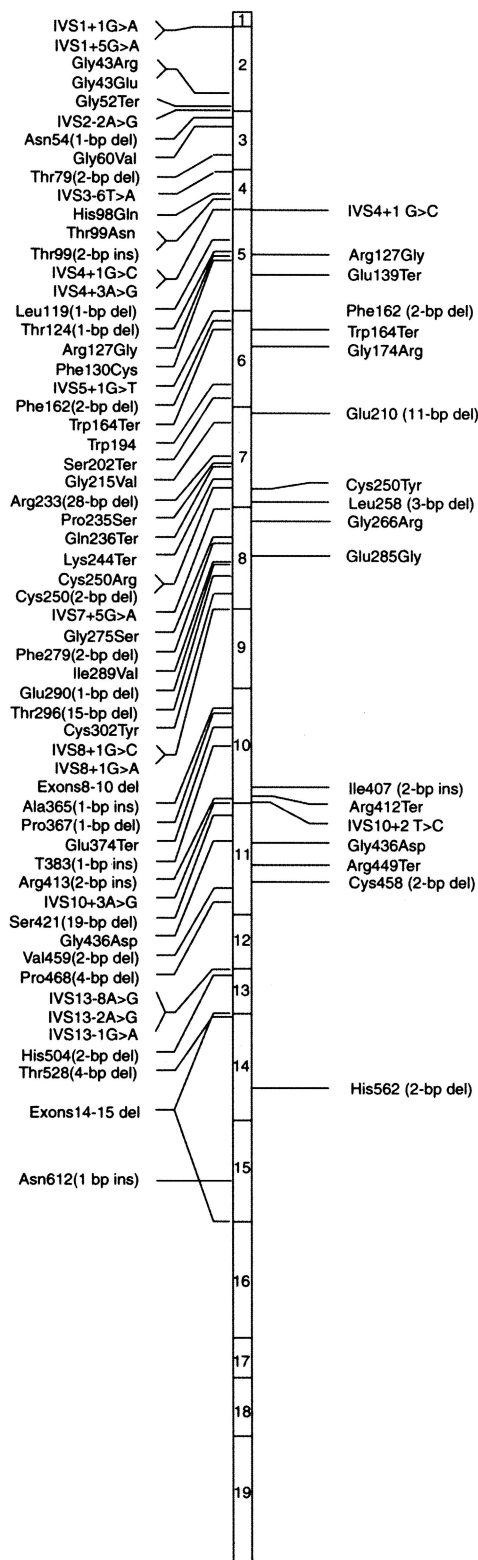


Figure 2 Location of all known mutations in *RPGR*. The right side designates mutations identified in this screen; all previously reported mutations are shown on the left.

Table 4
Mutations in *RPGR-ORF15* in Affected Hemizygototes with XLRP

Mutation ^a	Protein Change ^b	No. of Patients Affected
Deletion:		
432del	Glu146fsTer232	1
481-2del	Arg162fsTer184	9
481-4del	Arg162fsTer231	1
500-3del	Lys168fsTer231	1
504-7del	Gly170fsTer231	1
571-2del	Arg192fsTer250	1
632del	Glu212fsTer231	1
651-2del	Glu219fsTer250	6
653-4del	Glu219fsTer250	1
659-670del	Glu223fsTer250	1
673-4del	Glu226fsTer250	2
690-3del	Glu232fsTer235	3
1183-4del	Glu397fsTer495	1
1243-4del	Glu417fsTer495	1
1338-9del	Glu448fsTer495	1
Insertion:		
186ins, 5bp	Ala63fsTer113	1
353-461dup		1
Nonsense:		
370G→T	Glu125Ter	1
394G→T	Glu133Ter	1
424G→A	Glu143Ter	3
508G→T	Glu171Ter	3

^a Nucleotide positions and nomenclature are based on the work of Vervoort et al. (2000).

^b Frameshift mutations are designated according to the following example: Glu146fsTer232 refers to a frameshift mutation, in which Glu146 is the first amino acid altered, with termination of the open reading frame at residue 232.

provide essential information for the development of genotype-phenotype correlations for this heterogeneous disease. Together with our previous studies (Buraczynska et al. 1997; Fujita et al. 1997; Mears et al. 1999), we have performed a systematic analysis of 234 independent probands with a confirmed diagnosis of RP and probable X-linked recessive inheritance and have identified potential disease-causing mutations in approximately half of the families. Several of the reported mutations in the two genes are novel (see figs. 1, 2, and 3). Almost all of the newly discovered mutations are localized in the cofactor C homology domain of RP2 or in the RLD or exon ORF15 of RPGR, illustrating their functional significance.

In our studies, the observed mutation frequency in *RP2* and original *RPGR* exons 1–19 is 6% and 15%, respectively, and thus is in concordance with previous mutation screens (Buraczynska et al. 1997; Fujita et al. 1997; Hardcastle et al. 1999; Mears et al. 1999; Sharon et al. 2000). Surprisingly, however, we could identify *RPGR-*

ORF15 mutations in only 22% of our cohort, which is considerably lower than the published report of 60% (Vervoort et al. 2000). Interestingly, no causative mutations were detected in the 5' upstream regions of the two genes. Although all the probands in our study cohort carry a diagnosis of typical RP, the nature of several family structures, variations in disease manifestation in carrier females, and clinical phenotypes might support an autosomal pattern of inheritance in some of the families. Consequently we divided our cohort into categories defined by the evidence supporting X-linked inheritance (table 5). In category A, where the evidence for X-linked inheritance was the strongest, causative mutations were identified in 62% of patients (7% in *RP2*, 25% in *RPGR*,

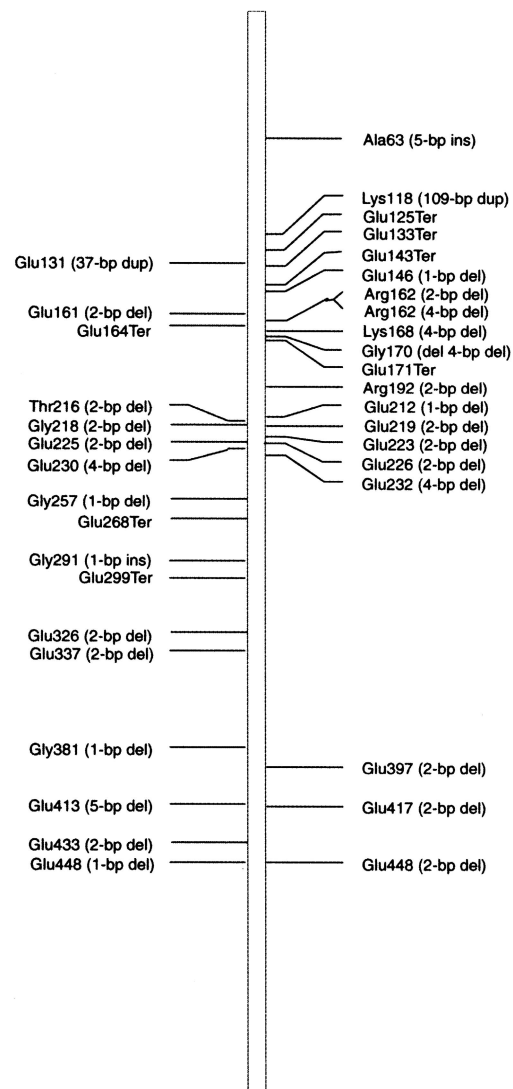


Figure 3 Location of all known mutations in *RPGR-ORF15*. The right side designates mutations identified in this screen; all mutations reported by Vervoort et al. (2000) are shown on the left.

Table 5
Distribution of Mutations in the North American XLRP Cohort

CATEGORY	NO. OF FAMILIES	NO. (%) OF MUTATIONS FOUND IN			
		<i>RP2</i>	<i>RPGR</i>	<i>ORF15</i>	Total
A	91	6 (7)	23 (25)	27 (30)	56 (62)
B	88	6 (7)	6 (7)	16 (18)	26 (32)
C	55	3 (5)	5 (9)	8 (15)	16 (29)
Total	234	15 (6)	34 (15)	51 (22)	100 (43)
Clinician-referred	160	12 (8)	26 (16)	42 (26)	80 (50)
RPRC-referred	74	3 (4)	8 (11)	9 (12)	20 (27)

and 30% in *RPGR-ORF15*). The disease locus in several category A families has been tightly linked to *RP3*; however, this screen failed to identify causative *RPGR* mutations (Fujita et al. 1997; D. K. Breuer, B. M. Yashar, and A. Swaroop, unpublished data). In class B, only 32% of the patients had mutations (7% in *RP2*, 7% in *RPGR*, and 18% in *RPGR-ORF15*). This frequency is similar to class C (single affected males) where 29% of patients have mutations (7% in *RP2*, 7% in *RPGR*, and 18% in *RPGR-ORF15*). The latter finding is particularly significant since it suggests that mutations in *RP2* and *RPGR* may account for disease in a considerable proportion of simplex cases that account for almost 50% of RP. We estimate that *RPGR* mutations would therefore be responsible for 15%–20% of all cases of RP, higher than any other single genetic locus.

Several possibilities can be put forward to explain why no mutation has been detected in almost 40% of even class A XLRP patients.

1. We examined >300 bp of the 5' upstream regions of the *RP2* and *RPGR* genes. Sequence changes in further upstream promoter or enhancer elements may affect gene expression.

2. Disease-associated mutations present within intronic sequences may alter the splicing pattern. Mutations at the branch-point are known to result in defective splicing in the *FBN2* gene (Maslen et al. 1997).

3. *RPGR* is a large and highly complex gene in which new exons continue to be identified (Kirschner et al. 1999; Vervoort et al. 2000). We had also screened another reported exon 15a (Kirschner et al. 1999) in 43 families, but we did not detect any sequence variation. An additional exon, *ORF14*, was identified in the same analysis that yielded *ORF15* (Vervoort et al. 2000); however, it was not screened in this study, since a previous study found no evidence for disease mutations. Another mutational hotspot in an as-yet-unidentified *RPGR* exon remains a possibility.

4. Another gene tightly linked to the region offers an alternative scenario. *RP6*, a locus for which the gene has yet to be cloned, is located <5 cM from *RP3* (Breuer et al. 2001) and is a valid candidate for future investigations.

The findings in this study may reflect a population difference, with the disease in our North American cohort attributable to a mutational hotspot in another exon of *RPGR* or another gene.

Since the vast majority of *RPGR* or *RP2* mutations are predicted to result in a truncated protein product, our study strongly argues that the XLRP disease state is caused by the loss of function of *RP2* or *RPGR*. This finding has significant implications for genetic diagnosis and gene-based therapy. The utility of a genetic test is dependent on specificity (the frequency with which the test yields a negative result when the disease is absent) and sensitivity (the frequency with which the test yields a positive result when the disease is present) (Gelehrter et al. 1998). Because of extensive alternative splicing and a lack of identifiable *RPGR* mutations even in genetically-linked *RP3* families, molecular testing by direct sequencing will have low specificity; nevertheless, it might be possible to greatly enhance the informativeness of testing by analyzing protein products in lymphocytes of at-risk males and carrier females. Protein-truncation tests have been applied successfully in studies of the *BRCA1* gene for breast cancer, the *APC* gene for colon cancer, and the *CHM* gene mutated in choroideremia (MacDonald et al. 1998; Balhausen 2000; Moore et al. 2000). With suitable *RPGR*- or *RP2*-specific antibodies, it may also be possible to identify mutations in the remainder of our cohort, if such changes alter the protein structure. We also suggest that a gene-based therapy that delivers a wild-type *RPGR* or *RP2* gene to the retina might be sufficient to correct the gene defect. Early applications of gene therapy to the treatment of retinopathies have provided strong proof of principle (Bennett and Maguire 2000; Hauswirth and Beaufre 2000; Bessant et al. 2001). A successful movement of this approach from research to clinical application will also depend on understanding the kinetics of the retinal degenerative process and administering the therapy early in the disease progression, while there are still viable photoreceptors. XLRP, like most genetic diseases, displays inter- and intrafamilial variability in the average age at onset of disease (Bird 1975; Fishman et al. 1988). Early molecular diagnosis of at-risk males should allow identification of the best patient candidates for therapeutic intervention. In summary, our study offers an important molecular definition of the XLRP population in North America and lays the groundwork for the future development of diagnostic testing and gene-based therapies for XLRP.

Acknowledgments

The authors sincerely appreciate the participation of the patients and the families. We wish to acknowledge a number of

clinicians, researchers, and genetic counselors who assisted in various aspects of this study; these include Drs. Kirk Alek, Sten Andreasson, Jean Bennett, Monika Buraczynska, Ricardo Fujita, Jacquie Greenberg, Albert Maguire, Michael Marmur, Helen Mintz-Hittner, Rajkumar Ramesar, and Marcela Pena; Ms. Janice Edwards; Mr. Eric Krivchenia; Ms. Kathleen O'Brien; Ms. Gina Osland; and Ms. Diana Wheaton. Special thanks are due to The Foundation Fighting Blindness, of Owings Mills, MD, for supporting these investigations. This research was also supported, in part, by the National Institutes of Health (grants EY07961, EY07003, EY05627, and EY05235), the Food and Drug Administration (grant FDR-001232), Research to Prevent Blindness, the Grant Healthcare Foundation, the Le Bonheur Children's Medical Center Research Program, the British Retinitis Pigmentosa Society, and the Scientific and Technical Research Council of the Turkish Republic (TUBITAK) and the State Planning Organization of Turkey (DPT) (grant 00K120660).

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

National Center for Biotechnology Information, <http://www4.ncbi.nlm.nih.gov/> (for descriptions of genes and sequences)
 Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for RP2 [MIM 312600], RP3 [MIM 312610], RP6 [MIM312612], RP24 [MIM 300155], and XLRP [MIM 26800])
 RetNet, <http://www.sph.uth.tmc.edu/Retnet/> (for map locations of retinal disease loci and genes)

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