Spectrum of Mutations in the *RPGR* Gene That Are Identified in 20% of Families with X-Linked Retinitis Pigmentosa

Monika Buraczynska,[1,*] Weiping Wu,[1] Ricardo Fujita,[1] Kinga Buraczynska,[1] Ellen Phelps,[1] Sten Andréasson,[3] Jean Bennett,[4,5] David G. Birch,[6] Gerald A. Fishman,[7] Dennis R. Hoffman,[6] George Inana,[8] Samuel G. Jacobson,[4] Maria A. Musarella,[9,**] Paul A. Sieving,[1] and Anand Swaroop[1,2]

Departments of [1]Ophthalmology and [2]Human Genetics, W.K. Kellogg Eye Center, University of Michigan, Ann Arbor; [3]Department of Ophthalmology, University of Lund, Lund, Sweden; [4]Department of Ophthalmology, Scheie Eye Institute, and [5]Institute for Human Gene Therapy, University of Pennsylvania, Philadelphia; [6]Retina Foundation of the Southwest, Dallas; [7]University of Illinois Eye and Ear Infirmary, Chicago; [8]Bascom Palmer Eye Institute, University of Miami School of Medicine, Miami; and [9]Departments of Ophthalmology, Pediatrics, and Genetics, The Hospital for Sick Children, University of Toronto, Toronto

Summary

The *RPGR* (*r*etinitis *p*igmentosa *G*TPase *r*egulator) gene for *RP3,* the most frequent genetic subtype of X-linked retinitis pigmentosa (XLRP), has been shown to be mutated in 10%–15% of European XLRP patients. We have examined the *RPGR* gene for mutations in a cohort of 80 affected males from apparently unrelated XLRP families, by direct sequencing of the PCR-amplified products from the genomic DNA. Fifteen different putative disease-causing mutations were identified in 17 of the 80 families; these include four nonsense mutations, one missense mutation, six microdeletions, and four intronic-sequence substitutions resulting in splice defects. Most of the mutations were detected in the conserved N-terminal region of the RPGR protein, containing tandem repeats homologous to those present in the RCC-1 protein (a guanine nucleotide-exchange factor for Ran-GTPase). Our results indicate that mutations either in as yet uncharacterized sequences of the *RPGR* gene or in another gene located in its vicinity may be a more frequent cause of XLRP. The reported studies will be beneficial in establishing genotype-phenotype correlations and should lead to further investigations seeking to understand the mechanism of disease pathogenesis.

Received July 24, 1997; accepted September 30, 1997; electronically published November 21, 1997.

Address for correspondence and reprints: Dr. Anand Swaroop, W.K. Kellogg Eye Center, University of Michigan, 1000 Wall Street, Ann Arbor, MI 48105. E-mail: swaroop@umich.edu

*On a sabbatical leave from Department of Medicine, Medical School, Lublin, Poland.

**Present affiliation: Department of Ophthalmology, SUNY Brooklyn Health Science Center, Brooklyn.

0002-9297/97/6106-0012$02.00

Introduction

X-linked retinitis pigmentosa (XLRP) is a severe form of retinal degeneration with an early onset of night blindness and progressive reduction of the visual field (Bird 1975; Fishman et al. 1988). Two major XLRP loci have been mapped: *RP2* (to Xp11.3-p11.23) and *RP3* (at Xp21.1) (for a review, see Aldred et al. 1994; Fujita and Swaroop 1996). The proportion of these loci varies in different populations; however, *RP3* seems to be more frequent, accounting for 60%–90% of affected pedigrees (Musarella et al. 1990; Ott et al. 1990; Teague et al. 1994; Fujita et al. 1997). Complete sequencing of the genomic region spanning the deletions in two XLRP patients led to the cloning of the *RPGR* (*r*etinitis *p*igmentosa *G*TPase *r*egulator) gene, which was shown to be mutated in 10%–15% of European XLRP families (Meindl et al. 1996; Roepman et al. 1996). The *RPGR* gene encodes a putative protein of 815 amino acids, with six tandem-repeat units in the amino-terminal region that have high homology to the repeats present in the RCC-1 protein (Ohtsubo et al. 1987), a guanine nucleotide-exchange factor (GEF) for Ran-GTPase (Drivas et al. 1990).

The goal of the present study was to elucidate the spectrum of *RPGR* mutations responsible for XLRP. Here we report a comprehensive analysis of the *RPGR* gene in 80 patients from independent XLRP families.

Subjects and Methods

Details of research procedures are similar to those recently described elsewhere (Fujita et al. 1997), and human subjects' participation was in accordance with institutional guidelines and the Declaration of Helsinki. The sequences of RPGR primers used for reverse-transcriptase–PCR (RT-PCR) analysis in this study are as follows: F5 (exon 3, sense), 5′-GAT TAG GAT CAA

Table 1

***RPGR* Mutations in Patients with XLRP**

Mutation[a,b]	Patient(s)	Exon(s)/IVS[b]	Nucleotide Change[a]	Effect of Mutation	Meiosis/Meioses Examined
Nonsense:					
G52X	A520	Exon 2	G→T at 213	Gly→stop	4
W164X	A101	Exon 6	G→A at 551	Trp→stop	4
Q236X	A224	Exon 7	C→T at 765	Gln→stop	1
E374X	A863	Exon 10	G→T at 1179	Glu→stop	3
Missense:					
G60V	A507, A910	Exon 3	G→T at 238	Gly→Val	4, 1
Deletion:					
430delC	A434	Exon 5	C at 430	Frameshift	1
928delA	A109, A463	Exon 8	A at 928	Frameshift	2, 1
Deletion of exons 8–10	A380	Exons 8-10	Exons 8–10	Splice defect and frameshift	2
del1320/1338	A567	Exon 11	19 bp at 1320–1338	Frameshift	5
del1571/1572	A924	Exon 13	CA at 1571–1572	Frameshift	7
del1641/1644	A662	Exon 14	ACAA at 1641–1644	Frameshift	2
Splicing:					
IVS4+3	A779	IVS4 donor site	A→G at 369+3	Exon 4 skipping, in-frame deletion	1
IVS7+5	A387	IVS7 donor site	G→A at 837+5	Exon 7 skipping, in-frame deletion	1

[a] Numbered according to Meindl et al. (1996).
[b] IVS = intervening sequence (intron).

AGT CAG CCA TC-3′; F2 (exon 5, sense), 5′-GGT GGA AAT AAT GAA GGA CAG TTG G-3′; B3 (exon 6, antisense), 5′-GGT CAC TTG CTG AGG GAC ACA G-3′; and B8 (exon 9, antisense), 5′-CGT GGC GAC CAT CTC CAA AAG-3′.

Results

We have examined the *RPGR* gene for mutations in genomic DNA derived from 80 affected males from a cohort of apparently unrelated XLRP families (68 North American and 12 Swedish). The genomic DNA was used to amplify exons 2–19 of the *RPGR* gene (corresponding to >98% of the coding region) and their flanking intronic sequences (for primers used, see Meindl et al. 1996; for methods, see Fujita et al. 1997). The primer set for the reported exon 1 (Meindl et al. 1996) was not used, because of inconsistent results of PCR amplification. PCR products were directly sequenced, and the sequence was compared with the normal *RPGR* gene. Fifteen different putative disease-causing mutations were identified in 17 of the 80 families; these include four nonsense mutations, one missense mutation, six microdeletions, and four intronic-sequence substitutions resulting in splice defects. All 17 mutations cosegregated with the disease in appropriate family members that were available for the study. Mutations reported here are designated according to the Ad Hoc Committee on Mutation Nomenclature (1996).

Nonsense Mutations

Single-base substitutions that result in premature termination codons were identified in four XLRP patients (table 1). All of these mutations are expected to produce a truncated and probably nonfunctional RPGR protein.

Missense Mutation

A change of Gly codon 60 (GGC) to a Val codon (GTC) was detected in two apparently unrelated patients (see table 1) but not in 100 normal and 78 other XLRP chromosomes. The Gly60 residue is conserved in the mouse Rpgr protein (D. Yan and A. Swaroop, unpublished data) and in RCC1 proteins from different species (Meindl et al. 1996). This suggests that G60V is a causative mutation. The G60V mutation has also been reported in another group of XLRP families (Bruns et al. 1997).

Two additional exonic variations were identified in one XLRP patient each: (i) A→G at nucleotide 282 in exon 3 and (ii) C→G at nucleotide 844 in exon 8. The A→G change is not detected in 71 normal and 79 other examined XLRP chromosomes, whereas the C→G change is not present in 79 other examined XLRP chromosomes. Since both changes result in conservative amino acid substitutions (I75V and A262G), these are probably not causative mutations.

Deletion Mutations

In seven XLRP patients, six different intragenic deletions were identified within the coding sequence of the *RPGR* gene (see table 1). A typical representation of our mutation analysis is provided in figure 1, which shows a 19-bp deletion of nucleotides 1320–1338 in exon 11 of one family. This deletion would result in a frameshift and premature stop codon. In another family, exons 8–10 could not be amplified from the genomic DNA of the proband (A380). Sequencing of the total RT-PCR products obtained from lymphocyte RNA of this proband confirmed the deletion of exons 8–l0 and revealed an aberrant splicing of the RPGR transcript, apparently induced by the deletion (data not shown).

Splice-Site Mutations

Mutations at the splice sites were identified in four patients. Two of the nucleotide substitutions were observed at the 5′ splice-donor sites (IVS4+3 and IVS7+5; see table 1) and cosegregated with the disease in the respective families. Sequencing of the total RT-PCR products spanning the respective exonic regions showed that both sequence changes resulted in exon skipping and, consequently, in in-frame deletion of an RCC1-homology repeat (data not shown). IVS4+3 and IVS7+5 are, therefore, expected to be causative mutations. Two other splice-site mutations (IVS10+3 and IVS13-8)—and their effect on the *RPGR* gene product—have been described elsewhere (Fujita et al. 1997).

Polymorphisms and Sequence Variations

Several polymorphisms (summarized in table 2) were observed during the mutation search. These sequence variations were detected in both affected and unaffected individuals and accounted for >2% of the examined population. The two sequence variations in exon 11 have been reported previously, by Roepman et al. (1996), in unaffected individuals and produce conservative amino acid substitutions. In our study, these two changes cosegregated in the population and were detected in seven patients. In addition, three intronic changes were identified in the variant sequence regions farther from the splice sites: (i) a G→A substitution in the intron 1 splice-acceptor region, IVS1-15 (detected in nine patients); (ii) an A→G substitution in the intron 10 splice-donor region, IVS10+16 (detected in five patients); and (iii) insertion of one nucleotide (A) in the intron 15 splice-donor region, after IVS15+17 (in one patient). The possible effect of these changes on splicing has not been determined.

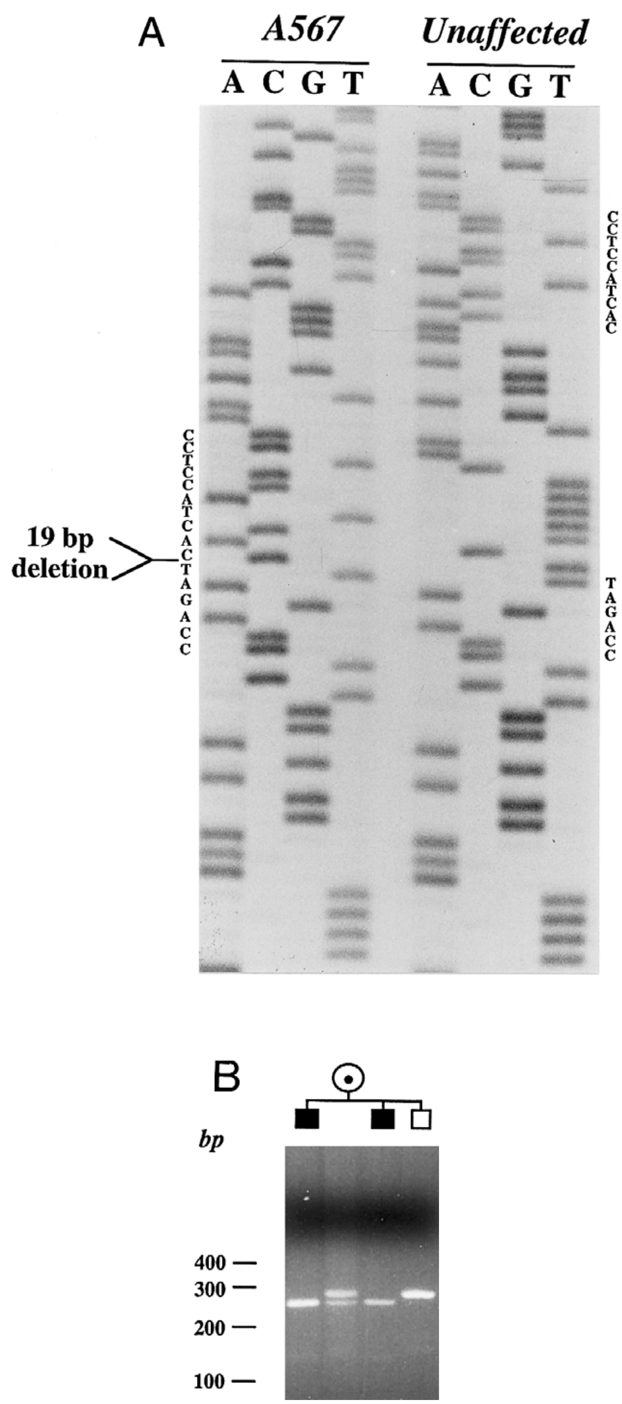

Figure 1 *A*, Genomic sequence showing a 19-bp deletion in exon 11 of the *RPGR* gene in patient A567 (family XLRP-236). For comparison, the uninterrupted sequence in an unaffected individual is also shown. *B*, PCR-amplified products of *RPGR* exon 11, showing segregation of the 19-bp deletion in the XLRP-236 family. A smaller product is observed in two affected brothers (*blackened squares*) and their heterozygous mother (*circle with a black dot*), whereas the unaffected male sibling (*unblackened square*) shows the product of correct size.

Table 2

***RPGR* Sequence Variations Detected in XLRP Patients**

Nucleotide Change[a]	Location	Effect on Coding Sequence
G or A at 1223	Exon 10	Silent (codon 388 Ala)
G or A at 1333	Exon 11	Conservative substitution (codon 425; Arg→Lys)
A or G at 1350	Exon 11	Conservative substitution (codon 431; Ile→Val)
G or A at 1756	Exon 14	Substitution (codon 566 Gly→Glu)
G or A at 87−15	IVS1-15	Not determined
T or C at 1566−68	IVS12-68	Not determined
T or C at 1566−97	IVS12-97	Not determined
A or G at 1631+11	IVS13+11	Not determined
T or C at 2300+11	IVS18+11	Not determined

[a] The first nucleotide shown is more common in the population tested.

Discussion

A majority of *RPGR* mutations were found to be unique to a single family, although one missense mutation and one single-base-pair deletion were detected in presumably unrelated patients (see table 1). One of the nonsense mutations (G52X) has been described elsewhere (Meindl et al. 1996), and the G60V missense mutation has also been reported (Bruns et al. 1997). Detection of a large number of independent mutations (even in a mostly North American population) suggests a high new-mutation rate of the *RPGR* gene and little or no founder effect. Preliminary linkage-disequilibrium studies with several polymorphic markers in the RP3 region also showed distinct haplotypes in most of the studied patients, providing evidence against a common origin of most XLRP mutations (R. Fujita and A. Swaroop, unpublished data).

The locations of the 15 different mutations identified in this study and of the 12 mutations reported elsewhere (Meindl et al. 1996; Roepman et al. 1996) are shown in figure 2. Most of the mutations were detected in the conserved N-terminal region of the RPGR protein, containing tandem repeats homologous to those present in the RCC-1 protein. Our analysis validates both the suggested functional importance of this region and a possible GEF function of the *RPGR* gene product. It should also be noted that the three in-frame deletions caused by splice defects remove one of the six RCC1-homology repeats from the resulting RPGR protein (for repeat units, see Meindl et al. 1996). Apparently, the loss of even one repeat may lead to XLRP. So far, no mutation has been identified in exons 16–19. Mutations in the C-terminal region encoded by these exons may not have a significant effect on protein function or may lead to diseases other than retinitis pigmentosa. The nature and location of *RPGR* mutations (in-frame deletions and missense mutations, in particular) should provide insights into the function of the RPGR protein by identifying critical domains/residues that, when altered, create functionally defective molecules.

In agreement with previous studies (Meindl et al. 1996; Roepman et al. 1996), we were able to demonstrate *RPGR* mutations in only 20% of the examined XLRP patients. One possible explanation for this low frequency can be the heterogeneity of disease genotype in the studied population. However, in 11 families in which the mutation could be localized to the RP3 region, only two causative mutations were detected (Fujita et al. 1997), and the RP3 subtype consistently accounts for

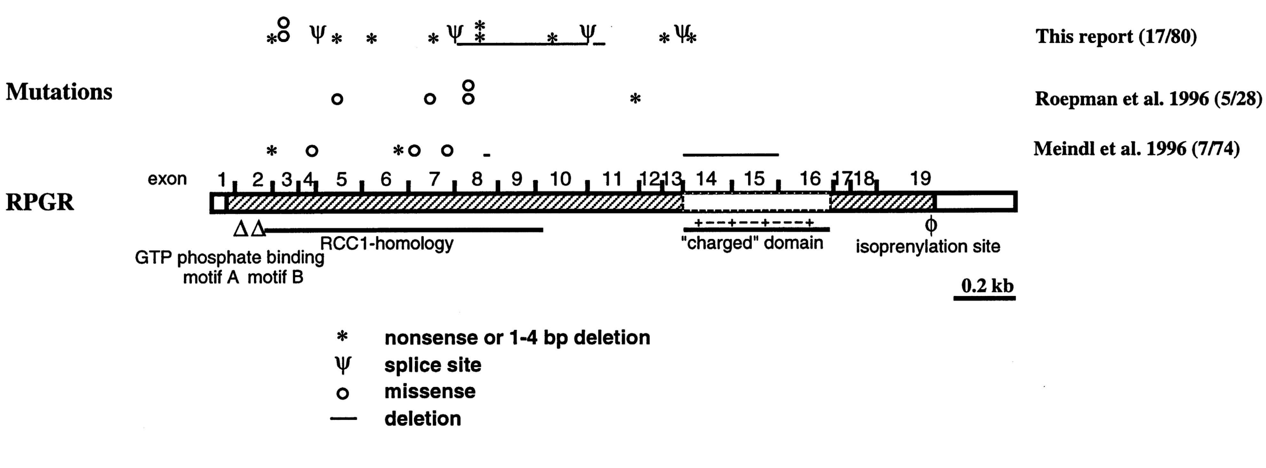

Figure 2 Schematic diagram of the *RPGR* cDNA, showing distribution of mutations identified in this report and others (Meindl et al. 1996; Roepman et al. 1996). Putative functional domains in the RPGR protein are indicated. The number of observed mutations/total number of families studied is given in parentheses, next to the reference.

60%–90% of genotyped XLRP pedigrees (Musarella et al. 1990; Ott et al. 1990; Teague et al. 1994; Fujita et al. 1997). It is possible that the *RPGR* gene contains as yet unidentified hotspots in sequences that have not been screened, such as the promoter region or intronic sequences and exon 1. Novel uncharacterized exons and/or inversions of large genomic regions (spanning complete exons) may also account for some of the remaining mutations. Alternatively, we cannot rule out additional genetic heterogeneity in XLRP—that is, the possibility that mutations in another gene, located in the proximity of *RPGR* at Xp21.1, also cause retinitis pigmentosa. Similar arguments have been advanced for other diseases—for example, X-linked ocular albinism and X-linked Alport syndrome—in which only one-third to one-half of the patients reveal mutations in the *OA1* and *COL4A5* collagen genes, respectively (Schiaffino et al. 1995; Knebelmann et al. 1996).

Different clinical presentations have been recognized in XLRP (Fishman et al. 1988). The reported studies will be beneficial in establishing correlation of *RPGR* mutations with phenotypic variations observed in hemizygous males and heterozygous carrier females in XLRP families. Because of their functional relevance, mutations in the RCC1-homology repeats are predicted to result in a relatively severe phenotype. Early attempts toward genotype-phenotype correlation have been initiated (Andréasson et al. 1997; Jacobson et al. 1997; Fishman et al., in press) Nonetheless, further investigations are required for an understanding of the mechanism of disease pathogenesis due to *RPGR* mutations.

Acknowledgments

We are grateful to the XLRP patients and their family members who participated in the study. We thank Drs. Kirk Alek, Jacquie Greenberg, John Heckenlively, and Albert Maguire, Ms. Gina Osland, and Ms. Janice Edwards, for some of the families included in mutation analysis. Thanks are also due to Cynthia Chen, Cara Coats, and T. J. Falls, for technical assistance, and to Dorothy Giebel, Jason Cook, and Mitch Gillett, for help in the preparation of the manuscript. This research was supported by the National Institutes of Health grants EY07961 (to A.S.), EY10820 (to J.B.), and EY05627 (to S.G.J.); by grants from The Foundation Fighting Blindness, Hunt Valley, MD (to A.S., D.G.B., G.A.F., M.A.M., P.A.S., and S.G.J.); by a grant from The RP Research Foundation of Canada (to M.A.M.); by a grant from The Chatlos Foundation, Inc. (to S.G.J.); by a grant from The Atkinson Charitable Foundation (to M.A.M.); and by an unrestricted grant from The Research to Prevent Blindness. We also acknowledge NIH grants EY07003 (CORE) and M01-RR00042 (General Clinical Research Center) and a Shared Equipment Grant from the Office of Vice President for Research. A.S. is recipient of a Research to Prevent Blindness Lew R. Wasserman Merit Award.

References

Ad Hoc Committee on Mutation Nomenclature (1996) Update on nomenclature for mutations. Hum Mutat 8:197–202

Aldred MA, Jay M, Wright AF (1994) X-linked retinitis pigmentosa. In: Wright AF, Jay B (eds) Molecular genetics of inherited eye disorders. Harwood Academic, Chur, Switzerland, pp 259–276

Andréasson S, Ponjavic V, Abrahamson M, Ehinger B, Wu W, Fujita R, Buraczynska M, et al (1997) Phenotypes in three Swedish families with X-linked retinitis pigmentosa caused by different mutations in the *RPGR* gene. Am J Ophthalmol 124:95–102

Bird AC (1975) X-linked retinitis pigmentosa. Br J Ophthalmol 59:177–199

Bruns G, Eisenman R, Dryja TP, Berson EL (1997) Mutation spectrum of the RPGR gene in X-linked retinitis pigmentosa. Invest Ophthalmol Vis Sci Suppl 38:A3178

Drivas GT, Shih A, Coutavas E, Rush MG, D'Eustachio P (1990) Characterization of four novel *ras*-like genes expressed in a human teratocarcinoma cell line. Mol Cell Biol 10:1793–1798

Fishman GA, Farber MD, Derlacki DJ (1988) X-linked retinitis pigmentosa—profile of clinical findings. Arch Ophthalmol 106:369–375

Fishman GA, Grover S, Buraczynska M, Wu W, Swaroop A. A new 2-base-pair deletion in the *RPGR* gene in an African-American family with X-linked retinitis pigmentosa. Arch Ophthalmol (in press)

Fujita R, Buraczynska M, Gieser L, Wu W, Forsythe P, Abrahamson M, Jacobson SG, et al (1997) Analysis of the *RPGR* gene in 11 pedigrees with the retinitis pigmentosa type 3 genotype: paucity of mutations in the coding region but splice defects in two families. Am J Hum Genet 61:571–580

Fujita R, Swaroop A (1996) RPGR: part one of the X-linked retinitis pigmentosa story. Mol Vis 2:4. Also available at http://www.emory.edu/MOLECULAR_VISION/v2/fujita

Jacobson SG, Buraczynska M, Milam AH, Chen C, Järvaläinen M, Fujita R, Wu W, et al. Disease expression in X-linked retinitis pigmentosa caused by a putative null mutation in the *RPGR* gene. Invest Ophthalmol Vis Sci 38:1983–1997

Knebelmann B, Breillat C, Forestier L, Arrondel C, Jacassier D, Giatras I, Drouot L, et al (1996) Spectrum of mutations in the COL4A5 collagen gene in X-linked Alport syndrome. Am J Hum Genet 59:1221–1232

Meindl A, Dry K, Herrmann K, Manson F, Ciccodicola A, Edgar A, Carvalho MRS, et al (1996) A gene (RPGR) with homology to the RCC1 guanine nucleotide exchange factor is mutated in X-linked retinitis pigmentosa (RP3). Nat Genet 13:35–42

Musarella MA, Anson-Cartwright L, Leal SM, Gilbert LD, Worton RG, Fishman GA, Ott J (1990) Multipoint linkage analysis and heterogeneity testing in 20 X-linked retinitis pigmentosa families. Genomics 8:286–296

Ohtsubo M, Kai R, Furno N, Sekiguchi T, Sekiguchi M, Hayashida H, Kei-chi K, et al (1987) Isolation and characterization of the active cDNA of the human cell cycle gene (RCC1) involved in the regulation of onset of chromosome condensation. Genes Dev 1:585–593

Ott J, Bhattacharya SS, Chen JD, Denton MJ, Donald J, Dubay

C, Farrar GJ, et al (1990) Localizing multiple X-chromosome-linked retinitis pigmentosa loci using multilocus homogeneity tests. Proc Natl Acad Sci USA 87:701–704

Roepman R, van Duijnhoven G, Rosenberg T, Pinckers AJLG, Bleeker-Wagemakers LM, Bergen AAB, Post J, et al (1996) Positional cloning of the gene for X-linked retinitis pigmentosa 3: homology with the guanine-nucleotide-exchange factor RCC1. Hum Mol Genet 5:1035–1041

Schiaffino MV, Bassi MT, Galli L, Renieri A, Bruttini M, De Nigris F, Bergen AAB, et al (1995) Analysis of the OA1 gene reveals mutations in only one-third of patients with X-linked ocular albinism. Hum Mol Genet 4:2319–2325

Teague PW, Aldred MA, Jay M, Dempster M, Harrison C, Carothers AD, Hardwick LJ, et al (1994) Heterogeneity analysis in 40 X-linked retinitis pigmentosa families. Am J Hum Genet 55:105–111