X-linked Retinitis Pigmentosa: *RPGR* Mutations in Most Families with Definite X Linkage and Clustering of Mutations in a Short Sequence Stretch of Exon ORF15

Ingrid Bader,[1] Oliver Brandau,[2,3] Helene Achatz,[2] Eckart Apfelstedt-Sylla,[4,5] Martin Hergersberg,[6] Birgit Lorenz,[7] Bernd Wissinger,[8] Bärbel Wittwer,[9] Günther Rudolph,[10] Alfons Meindl,[2] and Thomas Meitinger[1,11]

Purpose. A comprehensive screening was conducted for *RP2* and retinitis pigmentosa GTPase regulator (*RPGR*) gene mutations including *RPGR* exon ORF15 in 58 index patients. The frequency of *RPGR* mutations was assessed in families with definite X-linked recessive disease (xlRP), and a strategy for analyzing the highly repetitive mutational hot spot in exon ORF15 is provided.

Methods. Fifty-eight apparently unrelated index-patients were screened for mutations in all coding exons of the *RP2* and the *RPGR* genes, including splice-sites, by single-strand conformation polymorphism (SSCP) analysis, except for *RPGR* exon ORF15. A strategy for directly sequencing the large repetitive stretch of exon ORF15 from a 1.6-kb PCR-product was developed. According to pedigree size and evidence for X linkage, families were subdivided into three categories.

Results. Screening of 58 xlRP families revealed *RP2* mutations in 8% and *RPGR* mutations in 71% of families with definite X-linked inheritance. Mutations clustered within a ~500-bp stretch in exon ORF15. In-frame sequence alterations in exon ORF15 ranged from the deletion of 36 bp to the insertion of 75 bp.

Conclusions. Mutations in the *RPGR* gene are estimated to cause 15% to 20% of all cases of RP, higher than any other single RP locus. This report provides a detailed strategy to analyze the mutational hot spot in *RPGR* exon ORF15, which cannot be screened by standard procedures. The discrepancy of the proportion of families linked to the *RP3* locus and those having *RPGR* mutations is resolved in a subset of families with definite X linkage. (*Invest Ophthalmol Vis Sci.* 2003;44: 1458–1463) DOI:10.1167/iovs.02-0605

Retinitis pigmentosa (RP) is a genetically and clinically heterogeneous group of progressive photoreceptor degenerations with an overall incidence of 1 in 4000 in the general population. X-linked forms (xlRP, online Mendelian inheritance in man [OMIM 26800]) account for approximately 15% of RP cases and represent the most severe subtypes of this disease. They manifest themselves typically within the first two decades of life with night blindness and constriction of visual fields, progressing to severe visual loss or complete blindness by the third or fourth decade in affected men.[1,2] Five distinct RP loci on the X chromosome have been identified by linkage analyses predicting the loci *RP3* (OMIM 312619; Xp21.1) and *RP2* (OMIM 312600; Xp11.3) to account for 70% to 75% and 11% to 25% of all xlRP cases, respectively.[3] The *RP2* gene consists of five exons and codes for a predicted protein of 350 amino acids with domains homologous to cofactor C, to a porcine microtubule-associated protein (g-subunit of T-complex) and to a member of the nucleoside diphosphate kinase family.[4,5] It is mutated in 7% to 18% of different xlRP patient collectives. Mutations in the N terminus of the protein interfere with normal targeting to the plasma membrane.[3,6–8] Positional cloning of the *RPGR* gene (*RP3*) originally revealed a 2784-nucleotide (nt) ubiquitously expressed transcript that is organized in 19 exons coding for a predicted protein of 815 amino acids.[9]

Originally, this gene was found to be mutated in only 11% to 26% of patients with xlRP, with mutations being restricted to exons 1 to 15, which harbor a domain homologous to *RCC1* (regulator of chromosome condensation).[3,8,10] Using the *RCC1* homologous *RPGR* domain as a bait in yeast two-hybrid screens revealed two interacting proteins: the delta subunit of rod cyclic GMP phosphodiesterase (*PDE*-δ)[11] and the RPGR-interacting protein (*RPGRIP*) which localizes to the connecting cilium and is mutated in patients with Leber congenital amaurosis, a retinal dystrophy clinically related to RP.[12–15] Ectopic localization of cone opsins with subsequent cone and rod degeneration was observed in an *RPGR* knockout mouse.[16] Multiple 3′ splice variants were discovered in different tissues in humans, mice, cattle, and dogs, and two different mutations in exon ORF15 were detected in two distinct mutant dog strains with phenotypically distinguishable X-linked progressive retinal atrophy.[17–20] Recently, an alternatively spliced *RPGR* transcript containing a novel 1.7-kb 3′ terminal exon (ORF15) was identified. It is predominantly expressed in retina and harbors a mutational hot spot in patients with xlRP. This exon results from the retention of 1554 nt of the previously defined intron 15, includes a purine-rich repetitive region, and

From the [1]Institute of Human Genetics, National Research Center for Environment and Health (GSF), Neuherberg, Germany; the [2]Department of Medical Genetics at the University of Munich, Munich, Germany; the [4]University Eye Hospital Tübingen, Tübingen, Germany; the [6]Center for Laboratory Medicine, Cantonal Hospital Aarau, Aarau, Switzerland; the [7]Department of Paediatric Ophthalmology, Strabismology, and Ophthalmogenetics, Regensburg, Germany; the [8]Molecular Genetics Laboratory, University Eye Hospital Tübingen, Tübingen, Germany; the [9]Institute of Human Genetics, Münster, Germany; the [10]University Eye Clinic, Munich, Germany; and [11]GSF-Clinical Cooperation Group Ophthalmogenetics, Neuherberg, Germany.

Present affiliations: the [3]Department of Molecular Medicine, Max-Planck-Institute of Biochemistry, München-Martinsried, Germany; the [5]Eye Clinic, Katharinenhospital, Stuttgart, Germany.

Supported by European Union Grant BMH4-CT97-2341 (IB); German Ministry for Research and Education Grant KW9974 (AM); and German Research Foundation Grant DFG Lo 457/3,1-5 (BL).

Submitted for publication June 19, 2002; revised November 6, 2002; accepted November 14, 2002.

Disclosure: **I. Bader**, None; **O. Brandau**, None; **H. Achatz**, None; **E. Apfelstedt-Sylla**, None; **M. Hergersberg**, None; **B. Lorenz**, None; **B. Wissinger**, None; **B. Wittwer**, None; **G. Rudolph**, None; **A. Meindl**, None; **T. Meitinger**, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Ingrid Bader, Institute of Human Genetics, GSF–National Research Center for Environment and Health, Ingolstädter Landstrasse 1, D-85764 Neuherberg, Germany; ingrid.bader@gsf.de.

Table 1. Primer Sequences and Conditions for Analyzing *RPGR* Exon ORF15

Name	Sequence	Annealing °C (Cycles)	Length (Name)
PCR primers for analyzing exon ORF15*			
15F	5′ - CAGAGATCCTATCAGATGACC - 3′	60 (10); 58 (30)	1.6 kb
15aR3	5′ - TGTCTGACTGGCCATAATCG - 3′		
15F	5′ - CAGAGATCCTATCAGATGACC - 3′	58 (35)	237 bp (a)
15aR4	5′ - CCATTCTTCCTTCTCTGCTAG - 3′		
15aF4	5′ - GAGAATGAAAGGCAGGATGG - 3′	60 (35)	271 bp (b)
15aR3	5′ - TGTCTGACTGGCCATAATCG - 3′		

Name	Sequence	Sequencing Chemistry	Annealing (°C)	Extension (°C)	Additive (vol/vol)
Sequencing primers for the repetitive stretch†					
15aF3	5′ - GTAGAGGAGAAATGGAGAGG - 3′	dGTP-BDT	57	68	14%
15aF1	5′ - GAAGTGGAGGGAGAACGTG - 3′	dGTP-BDT	59	68	14%
TeS4	5′ - AAGGAGAAGGGGAAGGGGAGGAT - 3′	dGTP-BDT	59	68	14%
15aR5	5′ - GTTTGCCATATTTCACAGATCC - 3′	BDT	58	68	no
TER3	5′ - TCCTTCCTCCTCTTCCCCCTCCCA - 3′	dGTP-BDT	63	68	14%
400R10ib	5′ - CCTTCCTCCTCTTCCCCCTCA - 3′	BDT	58	63	5%

* Primers 15F and 15aR3 were used to amplify the 1.6-kb fragment which includes the repetitive stretch. PCR products a and b were screened by SSCP-analysis.

† Conditions for sequencing the repetitive stretch from the 1.6-kb fragment with nested primers.

codes for 567 C-terminal amino acids rich in glutamic acid and glycine residues. Exon ORF15 was mutated in 60% of xlRP families of mainly British and Irish descent,[19] in 18% to 30% of North American xlRP families,[3] in 32% of an unselected European xlRP population,[21] and in 15% of sporadic male patients with RP who have early onset of disease.[3] These data indicate that mutations in the *RPGR* gene may account for 15% to 20% of all cases of RP.[3] Mutations in exon ORF15 were also identified in patients with X-linked dominant RP[22] and in males with an X-linked cone–rod dystrophy phenotype.[23,24]

In this report, we present the results of a comprehensive screening for *RPGR* and *RP2* gene mutations in 58 xlRP-affected families and provide a strategy to screen the diagnostically most relevant *RPGR* exon ORF15.

Methods

Patients

The study, which involved human subjects, conformed to the tenets of the Declaration of Helsinki. Fifty-eight apparently unrelated index cases in members of clinically diagnosed xlRP families were screened for mutations in the *RP2* and the *RPGR* gene. Ophthalmic examination, including visual acuity, fundoscopy, Goldmann perimetry, dark-adaptation studies, and electroretinography (ERG), was performed in the affected males and in probable carrier females. The patients came from Germany ($n = 54$), Croatia ($n = 1$), Luxembourg ($n = 1$), Switzerland ($n = 1$), and Spain ($n = 1$). According to the size of the families, the number of affected males, and the number of carriers, three groups with variable probability of an X-linked recessive trait were defined: group I, families with at least two affected males in two generations connected by at least two obligate carrier females ($n = 24$); group II, families with affected males in two generations connected by a single carrier female ($n = 18$); group III, families with only two affected brothers or one affected male and a mother showing clinical signs confirming her carrier status ($n = 16$). Male-to-male transmission was absent in all pedigrees.

Mutation Screens in the *RPGR* Gene in Exons 1 to 19 and in the *RP2* Gene in Exons 1 to 5

The originally reported exons of the *RPGR* (exons 1–19) and the *RP2* gene (exons 1–5) were amplified from leukocyte DNA samples, as described by Meindl et al.[9] and Schwahn et al.,[4] and analyzed by single-strand conformation polymorphism (SSCP) analysis, using acrylamide gels (0.6× Serdogel; Serva, Heidelberg, Germany) running at 20°C with or without 10% glycerol in the gel matrix. Gels were stained with fluorescent dye (VistraGreen; Amersham, Freiburg, Germany), and bands were visualized with a fluorescence imager (FluorImager; Molecular Dynamics, Sunnyvale, CA). Products with aberrant SSCP patterns were column purified with a PCR purification kit (QIAquick; Qiagen, Hilden, Germany), sequenced using one of the SSCP PCR primers in a sequencing kit reaction (BigDye Terminator Cycle Sequencing Kit; Applied Biosystems, Foster City, CA) and analyzed on a DNA sequencer (Prism 377 DNA Sequencer; Applied Biosystems). All mutations were confirmed by sequencing a second PCR reaction.

Analysis of Exon ORF15 of the *RPGR* Gene

A strategy for directly sequencing the repetitive part of *RPGR* exon ORF15 with nested sequencing primers (Table 1) from a 1.6-kb PCR product was developed: the 1.6-kb PCR product was amplified by using primers (15F and 15aR3) situated outside the repetitive stretch in a total reaction volume of 100 μL containing 400 ng of genomic DNA, 20% (vol/vol) additive (Q-Solution; Qiagen), 0.2 μM of each primer, 200 μM of each deoxynucleotide, 1.5 U *Taq* DNA Polymerase, and 1× PCR buffer (Qiagen) with 2 mM $MgCl_2$. After an initial denaturation step of 7 minutes at 96°C, 10 PCR cycles were performed with denaturation at 96°C for 1 minute, annealing at 60°C for 1 minute, and extension at 72°C for 2 minutes, followed by 30 cycles with annealing at 58°C. In the final cycle, extension lasted for 7 minutes. PCR-products were purified as described and eluted from the column with 35 μL elution buffer. Thirteen microliters of the eluate was used as a template in a 30-μL sequencing reaction, using either of two sequencing kits (BigDye Terminator Cycle Sequencing, for the pyrimidine-rich strand, or dGTP BigDye Terminator Cycle Sequencing, for the purine-rich strand; Applied Biosystems), 0% to 14% (vol/vol) additive (Q-Solution; Qiagen), and 0.25 μM of one primer. Thirty sequencing cycles were performed with high annealing and extension temperatures (for details and primer sequences see Table 1). In the first cycle, denaturation lasted for 1 minute. Cycle reactions were then analyzed (Prism 3100 DNA Sequencer; Applied Biosystems). The less-repetitive flanking sequence parts were amplified separately with *Taq* polymerase (Ampli-*Taq* Gold DNA polymerase; Applied Biosystems) for PCR-product a and *Taq* DNA polymerase (Amersham) for PCR product b, according to the

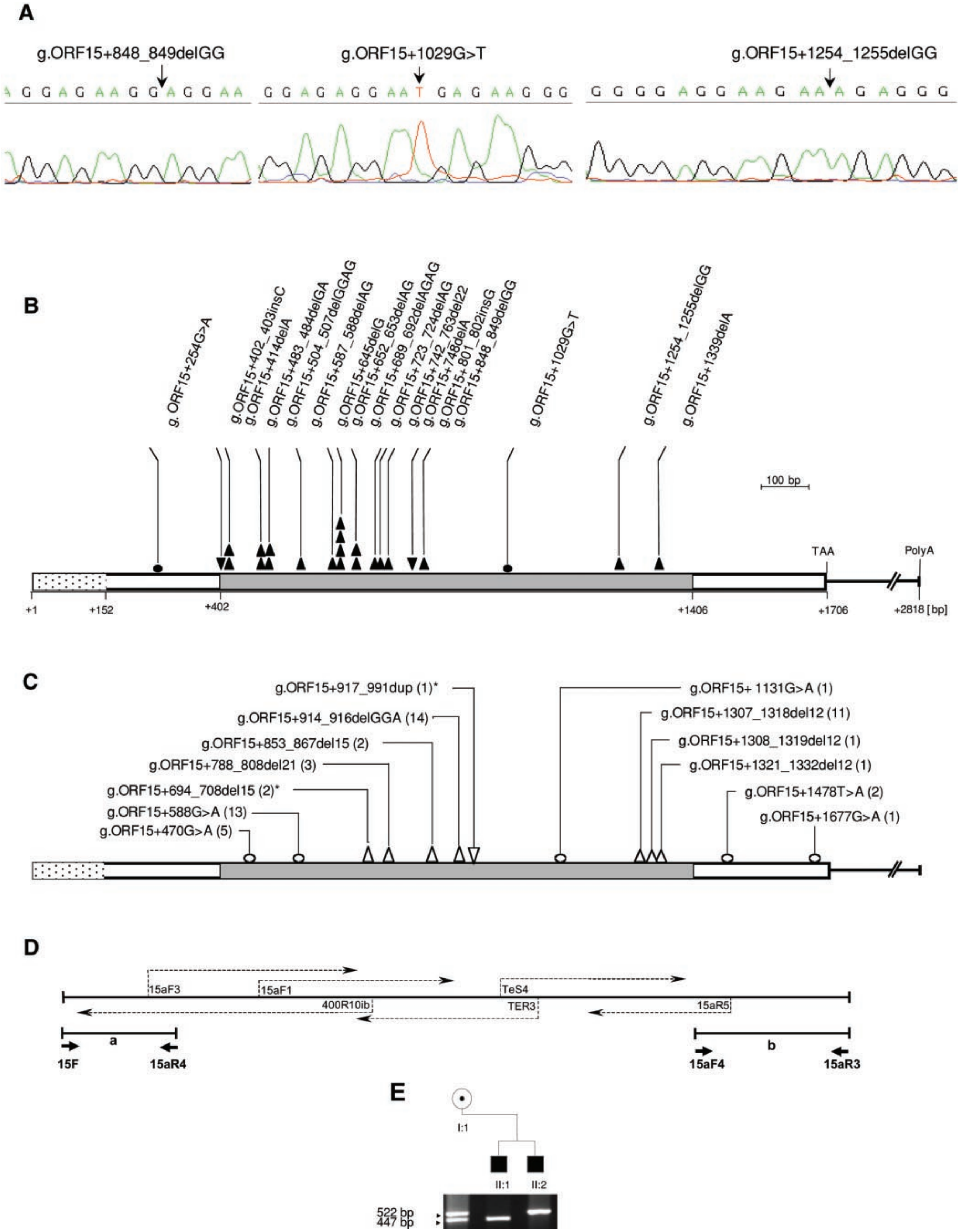
A
g.ORF15+848_849delGG
g.ORF15+1029G>T
g.ORF15+1254_1255delGG
B
g.ORF15+254G>A
g.ORF15+402_403insC
g.ORF15+414delA
g.ORF15+483_484delGA
g.ORF15+504_507delGGAG
g.ORF15+587_588delAG
g.ORF15+645delG
g.ORF15+652_653delAG
g.ORF15+689_692delAGAG
g.ORF15+723_724delAG
g.ORF15+742_763del22
g.ORF15+748delA
g.ORF15+801_802insG
g.ORF15+848_849delGG
g.ORF15+1029G>T
g.ORF15+1254_1255delGG
g.ORF15+1339delA
100 bp
TAA
PolyA
+1
+152
+402
+1406
+1706
+2818 [bp]
C
g.ORF15+917_991dup (1)*
g.ORF15+914_916delGGA (14)
g.ORF15+853_867del15 (2)
g.ORF15+788_808del21 (3)
g.ORF15+694_708del15 (2)*
g.ORF15+588G>A (13)
g.ORF15+470G>A (5)
g.ORF15+ 1131G>A (1)
g.ORF15+1307_1318del12 (11)
g.ORF15+1308_1319del12 (1)
g.ORF15+1321_1332del12 (1)
g.ORF15+1478T>A (2)
g.ORF15+1677G>A (1)
D
15aF3
15aF1
TeS4
400R10ib
TER3
15aR5
a
b
15F
15aR4
15aF4
15aR3
E
I:1
II:1
II:2
522 bp
447 bp

TABLE 2. *RP2* and *RPGR* mutations

Individual	Exon	DNA Mutation	Predicted Effect on Protein
RP2 gene mutations			
RP04/1759	1	Deletion of exon1	Truncated protein
RP28/1125	2	353G→A	R118H
XRP32/8801	2	352C→T	R118C
RPGR gene mutations in exons 1–15*			
RP67/5619	2	92delG	G12fsX67
XRP28/8805	5	514C→T	S152L
RP90/13532	6	677_680delAACA	Splice mutation, T207fsX221
RP87/11893	10	1275_1276delCT	L406fsX451
XRP3/8809†	15	1928_1929delAG	E624fsX628
RP07/122‡	2	213G→T	G52X
RP49/5474‡	7	703G→T	G215V
RP02/59‡	8	945_959del15bp	ΔTISYI296
RP22/4094	11–19	Deletion of exons 11–19	Truncated protein
RP16/489‡	14,15	Deletion of exons 14–15†	Truncated protein

* Nomenclature according to Meindl et al.[9]

† Annotation of this mutation according to Vervoort et al. is: g.ORF15+116_117delAG (p.ORF15E39fsX43).[19]

‡ The mutations in these patients have been reported previously.[9]

manufacturer's protocol. PCR-products were analyzed by SSCP and aberrantly migrating samples were sequenced according to standard methods, using one of the PCR-primers (Fig. 1D, Table 1). The sequence of primer 15aF5 (Fig. 1E) is 5′-AGTAGAGGGAGGGGAAGTAG-3′.

RESULTS

Thirty-six patients with xlRP from the present patient collective had been screened for mutations in *RPGR* exons 1 to 19 resulting in the detection of four different mutations.[9] Six additional mutations affecting exons 2, 5, 6, 10, 15, and 11 to 19 have now been found after screening exons 1 to 19 in a further 25 xlRP-affected families. None of these mutations has been reported before. Mutations affecting exons 16, 17, 18, or 19 exclusively were not detected. Altogether, mutations were found in 10 of the 58 xlRP-affected families by screening the originally reported *RPGR* gene exons (Table 2). Screening of the *RP2* gene revealed mutations in three of the 58 families, including a deletion mutation encompassing exon 1 that has not been reported so far (Table 2). Screening the large novel *RPGR* exon ORF15 in the remaining families revealed another 17 different mutations in 24 (41%) of the 58 families (Fig. 1B).

Segregation analysis of ORF15 mutations was performed in 14 independent families. The identified mutations were not present in 100 control chromosomes. Predominant mutations were frameshifts caused by 2-, 1-, and 4-bp deletions. Less common were 1-bp insertions and a single larger deletion (22 bp). Only two point mutations were detected, causing protein truncation by premature stop codons (Fig. 1C). Frameshift mutations clustered within a 448-bp stretch in exon ORF15 between g.ORF15+402-849, which contained 83% (20/24) of all mutations detected in exon ORF15. In-frame alterations which were all located within the most repetitive stretch of ORF15 ranged from the loss of 36 bp in chromosomes in which two different deletions coincided (g.ORF15+788_808del21 and g.ORF15+853_867del15) to the gain of 75 bp (Fig. 1E); They were detected in 34% of all the analyzed chromosomes, and most of them were present in 100 unaffected control chromosomes (Fig. 1C). Two in-frame alterations were found in patients only: First, the deletion g.ORF15+694_708del15 (p.ORF15+231_235delGGEVE) was present in two families that additionally harbored a disease causing frameshift mutation (g.ORF15+504_507delGGAG), indicating that those families may be related. Second, the in-frame duplication g.ORF15+917_991dup, which reduplicates three of four completely identical 24-bp repeat units, did not segregate with the disease in family RP15 (Fig. 1E).

DISCUSSION

Mutations in the *RPGR* gene are estimated to be responsible for 15% to 20% of all cases of RP, higher than any other single locus.[3] Two comprehensive mutational screens in xlRP-affected patients have been published to date with various results regarding the proportion of *RPGR* mutations detected in families with an X-linked recessive trait. Vervoort et al. found *RPGR* gene mutations in 72% of a patient collective of British and Irish descent, which is in accordance with linkage analyses. Of the mutations in xlRP families, 60% were found in exon ORF15.[19,25] In the first confirmatory screen *RPGR* mutations were found in 55% (exon ORF15: 30%) of families of North American decent with definite X linkage.[3] In a recent study a similar mutation detection rate (exon ORF15: 32%) was observed in an unselected European xlRP-affected patient collective.[21]

In the present study *RPGR* mutations were found in 71% and exon ORF15 mutations were present in 63% of families

FIGURE 1. (**A**) Three mutations in *RPGR* exon ORF15. (**B**) Distribution of 17 different mutations in *RPGR* exon ORF15, detected in 24 families. Deletions (▲), insertions (▼), point mutations (●). Two parts of exon ORF15 are indicated: exon 15 of the originally reported *RPGR* transcript (g.ORF15+1–152 ▨), and the repetitive purine-rich stretch (g.ORF15+402–1406 □) consisting of imperfect direct repeats of 6 to 33 bp. (**C**) Distribution and frequency (in parentheses) of sequence variations detected in 100 male control chromosomes. Two sequence variations (*) were present only in patients with xlRP patients. (**D**) Outline of the strategy for screening exon ORF15. PCR products a and b were screened using SSCP analysis, and the large 1.6-kb PCR product (*bold line*) was sequenced using the primers depicted (*dashed arrows*). (**E**) Segregation analysis in family RP15. The reduplication g.ORF15+917_991dup does not segregate with the disease. The insertion is present in the affected male II:2 and in his heterozygote mother, but not in the affected son II:1. A 447-bp PCR product generated with the primers 15aF5 and TER3 (Table 1) was separated on a 1.5% agarose gel. The reduplication is predicted to insert 16 glutamic acid and 9 glycine residues into the glutamic-acid–rich stretch.

Table 3. Mutations in the *RP2* and the *RPGR* Gene in Different Subsets of Families

xl Group	Patients	*RP2/RPGR* Gene Mutations	*RPGR* Gene Mutations: All	*RPGR* Gene Mutations: Exons 1–15	*RPGR* Gene Mutations: ORF15	*RP2* Gene Mutations	No *RP2/RPGR* Gene Mutation
I	24	19 (79)	17 (71)	2 (8)	15 (63)	2 (8)	5 (21)
II	18	10 (53)	9 (50)	2 (11)	7 (39)	1 (6)	8 (47)
III	16	8 (50)	8 (50)	6 (37)	2 (12)	0 (0)	8 (50)
Total	58	37 (64)	34 (59)	10 (17)	24 (41)	3 (5)	21 (36)

Data are the number of patients with the percentage of the total in the group in parentheses.

with definite X linkage (Table III). In the present study, the inclusion criteria for group I families (definite X linkage) differed from the criteria applied in the first confirmatory screen.[3] There had to be at least two obligate carrier females per pedigree (see the Methods section) to include only families with a pedigree structure that is comparable to those pedigrees generally used in linkage analyses (large multiplex families providing multiple informative meioses). In this subset of families, the discrepancy of the proportion of xlRP families linked to the *RP3* locus and those having *RPGR* mutations is resolved. The missing mutations reside in exon ORF15. We conclude from our data that not yet identified exons at the *RP3* locus are not likely to play a major role in xlRP.

Screening of exon ORF15 is technically challenging[25,26] because of its repetitive nature and because of the presence of in-frame deletions or duplications in 34% of the general population, the majority of which delete or duplicate one or more tandem repeats. The strategy provided in the present study allows sequencing of both strands of the repetitive stretch with nested primers from a single PCR product. This approach avoids mispriming and PCR artifacts, which may easily occur with the use of PCR primers situated within the repetitive stretch.

The substantial decrease of mutation detection rate in the *RPGR* and the *RP2* gene in families with smaller pedigrees (groups II and III, Table 3) may be due to unrecognized autosomal disease with reduced penetrance, as the possibility of an autosomal mode of inheritance cannot be ruled out with certainty. Unrecognized autosomal traits in smaller families may also be the reason for the lower mutation detection rate in the study screening families of North American descent with definite X linkage (definite X linkage: at least two generations of affected males that were related through an unaffected or carrier female)[3] and in a screening of unselected European xlRP families.[21]

In families with fewer carrier females (groups II and III) a low rate of exon ORF15 mutations contrasted with the relative high frequency of mutations in *RPGR* exons 1 to 14 in the present study. An increased mutability of exon ORF15 in the male germline resulting preferentially in pedigrees with multiple obligate carrier females would be a possible explanation for this bias. However, because our pedigree data do not clearly support this hypothesis and because DNA samples from the earlier family generations have not been collected, this could not be investigated further.

It is interesting that 83% of the pathogenic mutations (mainly frameshifts) in exon ORF15 were detected within a 447-bp stretch at the 5′ end of the most repetitive sequence. A similar clustering was observed in two previous comprehensive studies.[3,19] Mutation analysis therefore should be primarily focused on this 447-bp stretch.

Frameshifts occurring at the beginning of the purine-rich stretch (mutations: g.ORF15+652_653delAG, g.ORF15+742_763del22bp, g.ORF15+748delA, g.ORF15+801_802insG, g.ORF15+848_849delGG) create long open reading frames, due to the absence of T-nucleotides in the following 0.6 kb. The shifted open reading frames are predicted to code for 232-298 amino acid stretches rich in alkaline residues (arginine and/or lysine), reversing the charge of the polypeptide-stretch. Detailed genotype–phenotype analysis in these patients will be of use, as a mutant dog strain carrying a frameshift mutation that leads to 34 additional alkaline residues before protein truncation displays a severe phenotype that becomes manifest during retinal development with mutant protein aggregating in the endoplasmic reticulum.[20]

No mutations in the *RPGR* or *RP2* gene were detected in 20% of group I families. In these patients, undiscovered mutations may reside either in regulatory sequences of the *RPGR* or *RP2* gene or in one of the three additional xlRP loci (*RP6*, *RP23*, *RP24*) described to date.[27–29] SSCP may not identify all mutations. The average mutation detection rate of SSCP analysis using acrylamide gels without glycerol is reported to be in the range of 70% to 90%; with glycerol, it is reported to be approximately 68%.[30,31] Combination of the two conditions dramatically increases the sensitivity and produces detection frequencies above 90%.[30,32] In the present study, gels were examined under both conditions.

Recently the in-frame deletion g.ORF15+694_708del15 has been proposed to cause X-linked cone dystrophy in affected males of a single family where no other *RPGR* mutation was found.[33] It originally was supposed to be a benign sequence alteration because of the additional presence of a protein-truncating mutation (g.ORF15+897G→T) in one patient with xlRP.[19] The present study identified this in-frame deletion in two patients from different families that have, in addition, a protein-truncating mutation (g.ORF15+504_507delGGAG) distinct from the mutation found in the patient in the study by Vervoort et al.[19] This provides further evidence that the 15 bp in-frame deletion may instead be a rare sequence variant that does not cause xlRP or X-linked cone dystrophy. The in-frame duplication g.ORF15+917_991dup is the largest in-frame alteration detected in exon ORF15 to date. This reduplication is not the cause of xlRP in the described family (Fig. 1E) and is predicted to leave intact the acidic character of the glutamic-acid–rich domain. It is therefore interpreted as a rare benign sequence alteration, because it was not present in 100 control chromosomes.

In conclusion, the results of the present study demonstrate that the majority of *RPGR* mutations reside in exon ORF15 in families with definite X linkage. Together with previous studies,[3,19] they confirm the *RPGR* gene as the diagnostically most important single genetic locus in RP. Our data point to the high degree of sequence variation in RPGR exon ORF15 in the general population and indicate that the pathogenicity of rare in-frame sequence variations must be cautiously interpreted.

Acknowledgments

The authors thank the patients and families for participating in the study and Caecilia Haberger and Jack Favor for help with the language.

References

1. Bird AC. X-linked retinitis pigmentosa. *Br J Ophthalmol.* 1975;59: 177–199.
2. Fishman GA, Farber MD, Derlacki DJ. X-linked retinitis pigmentosa: profile of clinical findings. *Arch Ophthalmol.* 1988; 106:369–375.
3. Breuer DK, Yashar BM, Filippova E, et al. A comprehensive mutation analysis of RP2 and RPGR in a North American cohort of families with X-linked retinitis pigmentosa. *Am J Hum Genet.* 2002;70:1545–1554.
4. Schwahn U, Lenzner S, Dong J, et al. Positional cloning of the gene for X-linked retinitis pigmentosa 2. *Nat Genet.* 1998;19:327–332.
5. Miano MG, Testa F, Filippini F, et al. Identification of novel RP2 mutations in a subset of X-linked retinitis pigmentosa families and prediction of new domains. *Hum Mutat.* 2001;18:109–119.
6. Chapple JP, Hardcastle AJ, Grayson C, Spackman LA, Willison KR, Cheetham ME. Mutations in the N-terminus of the X-linked retinitis pigmentosa protein RP2 interfere with the normal targeting of the protein to the plasma membrane. *Hum Mol Genet.* 2000;9:1919–1926.
7. Schwahn U, Paland N, Techritz S, Lenzner S, Berger W. Mutations in the X-linked RP2 gene cause intracellular misrouting and loss of the protein. *Hum Mol Genet.* 2001;10:1177–1183.
8. Sharon D, Bruns GA, McGee TL, Sandberg MA, Berson EL, Dryja TP. X-linked retinitis pigmentosa: mutation spectrum of the RPGR and RP2 genes and correlation with visual function. *Invest Ophthalmol Vis Sci.* 2000;41:2712–2721.
9. Meindl A, Dry K, Herrmann K, et al. A gene (RPGR) with homology to the RCC1 guanine nucleotide exchange factor is mutated in X-linked retinitis pigmentosa (RP3). *Nat Genet.* 1996;13:35–42.
10. Roepman R, van Duijnhoven G, Rosenberg T, et al. Positional cloning of the gene for X-linked retinitis pigmentosa 3: homology with the guanine-nucleotide-exchange factor RCC1. *Hum Mol Genet.* 1996;5:1035–1041.
11. Linari M, Ueffing M, Manson F, Wright A, Meitinger T, Becker J. The retinitis pigmentosa GTPase regulator, RPGR, interacts with the delta subunit of rod cyclic GMP phosphodiesterase. *Proc Natl Acad Sci USA.* 1999;96:1315–1320.
12. Roepman R, Bernoud-Hubac N, Schick DE, et al. The retinitis pigmentosa GTPase regulator (RPGR) interacts with novel transport-like proteins in the outer segments of rod photoreceptors. *Hum Mol Genet.* 2000;9:2095–2105.
13. Boylan JP, Wright AF. Identification of a novel protein interacting with RPGR. *Hum Mol Genet.* 2000;9:2085–2093.
14. Hong DH, Yue G, Adamian M, Li T. Retinitis pigmentosa GTPase regulator (RPGRr)-interacting protein is stably associated with the photoreceptor ciliary axoneme and anchors RPGR to the connecting cilium. *J Biol Chem.* 2001;276:12091–12099.
15. Dryja TP, Adams SM, Grimsby JL, et al. Null RPGRIP1 alleles in patients with Leber congenital amaurosis. *Am J Hum Genet.* 2001; 68:1295–1298.
16. Hong DH, Pawlyk BS, Shang J, Sandberg MA, Berson EL, Li T. A retinitis pigmentosa GTPase regulator (RPGR)-deficient mouse model for X-linked retinitis pigmentosa (RP3). *Proc Natl Acad Sci USA.* 2000;97:3649–3654.
17. Kirschner R, Rosenberg T, Schultz-Heienbrok R, et al. RPGR transcription studies in mouse and human tissues reveal a retina-specific isoform that is disrupted in a patient with X-linked retinitis pigmentosa. *Hum Mol Genet.* 1999;8:1571–1578.
18. Zeiss CJ, Ray K, Acland GM, Aguirre GD. Mapping of X-linked progressive retinal atrophy (XLPRA), the canine homolog of retinitis pigmentosa 3 (RP3). *Hum Mol Genet.* 2000;9:531–537.
19. Vervoort R, Lennon A, Bird AC, et al. Mutational hot spot within a new RPGR exon in X-linked retinitis pigmentosa. *Nat Genet* 2000; 25:462–466.
20. Zhang Q, Acland GM, Wu WX, et al. Different RPGR exon ORF15 mutations in Canids provide insights into photoreceptor cell degeneration. *Hum Mol Genet.* 2002;11:993–1003.
21. Pusch CM, Broghammer M, Jurklies B, Besch D, Jacobi FK. Ten novel ORF15 mutations confirm mutational hot spot in the RPGR gene in European patients with X-linked retinitis pigmentosa. *Hum Mutat* 2002;20:405.
22. Rozet JM, Perrault I, Gigarel N, et al. Dominant X linked retinitis pigmentosa is frequently accounted for by truncating mutations in exon ORF15 of the RPGR gene. *J Med Genet.* 2002;39:284–285.
23. Mears AJ, Hiriyanna S, Vervoort R, et al. Remapping of the RP15 locus for X-linked cone-rod degeneration to Xp11.4-p21.1, and identification of a de novo insertion in the RPGR exon ORF15. *Am J Hum Genet.* 2000;67:1000–1003.
24. Demirci FY, Rigatti BW, Wen G, et al. X-linked cone-rod dystrophy (locus COD1): identification of mutations in RPGR exon ORF15. *Am J Hum Genet.* 2002;70:1049–1053.
25. Vervoort R, Wright AF. Mutations of RPGR in X-linked retinitis pigmentosa (RP3). *Hum Mutat.* 2002;19:486–500.
26. Yokoyama A, Maruiwa F, Hayakawa M, et al. Three novel mutations of the RPGR gene exon ORF15 in three Japanese families with X-linked retinitis pigmentosa. *Am J Med Genet.* 2001;104: 232–238.
27. Ott J, Bhattacharya S, Chen JD, et al. Localizing multiple X chromosome-linked retinitis pigmentosa loci using multilocus homogeneity tests. *Proc Natl Acad Sci USA.* 1990;87:701–704.
28. Gieser L, Fujita R, Goring HH, et al. A novel locus (RP24) for X-linked retinitis pigmentosa maps to Xq26–27. *Am J Hum Genet.* 1998;63:1439–1447.
29. Hardcastle AJ, Thiselton DL, Zito I, et al. Evidence for a new locus for X-linked retinitis pigmentosa (RP23). *Invest Ophthalmol Vis Sci.* 2000;41:2080–2086.
30. Ravnik-Glavac M, Glavac D, Dean M. Sensitivity of single-strand conformation polymorphism and heteroduplex method for mutation detection in the cystic fibrosis gene. *Hum Mol Genet.* 1994; 3:801–807.
31. Vidal-Puig A, Moller DE. Comparative sensitivity of alternative single-strand conformation polymorphism (SSCP) methods. *Biotechniques.* 1994;17:490–492, 494, 496.
32. Holinski-Feder E, Weiss M, Brandau O, et al. Mutation screening of the BTK gene in 56 families with X-linked agammaglobulinemia (XLA): 47 unique mutations without correlation to clinical course. *Pediatrics.* 1998;101:276–284.
33. Yang Z, Peachey NS, Moshfeghi DM, et al. Mutations in the RPGR gene cause X-linked cone dystrophy. *Hum Mol Genet.* 2002;11: 605–611.