

MUTATION IN BRIEF

Five Novel *RPGR* Mutations in Families with X-Linked Retinitis Pigmentosa

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X-linked forms of retinitis pigmentosa (XLRP) are among the most severe because of their early onset, often leading to significant visual impairment before the fourth decade. *RP3*, genetically localized at Xp21.1, accounts for 70% of XLRP in different populations. The *RPGR* (Retinitis Pigmentosa GTPase Regulator) gene that was isolated from the *RP3* region is mutated in 20% of North American families with XLRP. From mutation analysis of 27 independent XLRP families, we have identified five novel *RPGR* mutations in 5 of the families (160delA, 789 A>T, IVS8+1 G>C, 1147insT and 1366 G>A). One of these mutations was detected in a family from Chile. © 2000 Wiley-Liss, Inc.

KEY WORDS: retinitis pigmentosa; retinal degeneration; *RPGR*; *RP3*; XLRP

INTRODUCTION

Retinitis pigmentosa (RP) is a collective term for a group of phenotypically similar inherited progressive retinal degenerations. RP is genetically heterogeneous; more than thirty loci have been identified and they are transmitted as autosomal dominant, autosomal recessive and X-linked subtypes (<http://www.sph.uth.tmc.edu/Retnet>). The X-linked forms of RP (XLRP) are among the most severe because of their early onset, often leading to significant visual impairment before the fourth decade (Bird 1975; Fishman et al. 1988). There are two major loci for XLRP; *RP2* and *RP3*, which are clinically indistinguishable and map within 20 centimorgans (cM) at Xp. *RP3* accounts for 70% of XLRP (Ott et al. 1980) in different populations and was genetically localized at Xp21.1. The gene *RPGR* (Retinitis Pigmentosa GTPase Regulator; MIM# 312610) was isolated in the critical region and shown to be mutated in XLRP patients (Meindl et al. 1996; Roepman et al. 1996). Recently, Vervoort et al. (2000) described a

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new *RPGR* exon, called ORF15, in which they identified mutations in 60% of XLRP patients analyzed (the majority of which are of British and Irish origin). In their population, mutations have now been identified in over 75% of XLRP patients. In our previous studies of the North American population, only 20% of XLRP patients (even those with the *RP3* genotype) show *RPGR* mutations (Buraczynska et al. 1997; Fujita et al. 1997), but ORF15 has yet to be analyzed in this population, although one mutation has been reported in one North American family with X-linked cone-rod degeneration (Mears et al. 2000). The putative *RPGR* protein described in Meindl et al. (1996) has 815 amino acids encoded in a 2.8 kb transcript. Here, we describe five novel mutations in the *RPGR* gene in 5 of the 27 XLRP families that we screened for exons 1-19.

MATERIALS AND METHODS

XLRP Patients

RP status was established by history, fundus examination, visual field tests, and electroretinograms. The X-linked status was designated by inspection of the pedigrees, showing no male-to-male transmission and a milder phenotype in female carriers. Participating patients and their families were informed of the objectives of the studies, and procedures were performed in agreement with the Declaration of Helsinki and with our institutional guidelines. DNA was prepared from blood by standard procedures.

Mutation detection

RPGR exons were PCR amplified from genomic DNA using the previously described primers (Meindl et al. 1996). The products were sequenced manually using the Thermo Sequenase ³³P-Radiolabelled Terminator Cycle Sequencing kit (Amersham Pharmacia Biotech, New Jersey, USA). When available, DNA of family members were analyzed to confirm the segregation of mutations. RNA was extracted from blood using TRIzol Reagent (Life Technologies Inc., Maryland, USA). RT-PCR was performed with the ThermoScript RT-PCR System (Life Technologies Inc., Maryland, USA) using appropriate primers (Exon 3-forward primer 5'GATTAGGATCAAAGTCAGCCATC-3', Exon 14 reverse primer 5'TCGTTTTTCAGTAAGAGCTGTATCC-3'). The splicing error was detected by sequencing of the RT-PCR product with an internal primer (Exon 9 reverse primer 5'GTGGCGACCATCTCCAAAAG-3').

RESULTS

Direct sequencing of the *RPGR* gene (exons 1-19) revealed causative mutations in five of the 27 XLRP patients (representing 27 independent families) that were examined (Table 1).

Table 1: Summary of the *RPGR* mutations identified in this report.

Patient ID	Exon / Intron	Nucleotide sequence change*	Effect of Mutation
A316	Exon 2	160delA	Frameshift
A1014	Exon 7	789 A>T	K244X
A1021	Intron 8	IVS8+1 G>C	Splicing error, exon 8 skipped
A1066	Exon 10	1147insT	Frameshift
A1192	Exon 11	1366 G>A	G436D

* According to Genbank No. U57629

An intragenic deletion was detected in patient A316 causing the loss of nucleotide 160 in exon 2. This mutation results in a frameshift and premature stop thereby resulting in a truncated protein. This deletion segregated in phase with the disease in ten affected members of this family and seven obligate carriers.

In patient A1014, a transversion A>T occurred in nucleotide 789 in exon 7; this event changes the codon for lysine to a stop codon. The resulting putative protein would be truncated at the amino acid 243, missing about half of the functional RCC1-like repeats and all the C-terminal half including the charged domain and a putative isoprenylation site. This mutation was found in five affected XLRP males and two carriers of the same family.

In patient A1021, G>C transversion at the invariable first G of the intron splice donor site of intron 8 is predicted to result in a splicing defect. This mutation segregated in phase with the disease in 14 other members in the family. RT-PCR analysis of the lymphocyte total RNA from an affected male showed that the splice site mutation results in the skipping of *RPGR* exon 8 (data not shown).

In patient A1066, a T nucleotide insertion in a stretch of five Ts at position 1147 to 1151 in exon 10, results in a frameshift and premature truncation of the *RPGR* protein.

In patient A1192, a G>A transition at nucleotide 1366 in exon 11 results in a glycine to aspartic acid substitution. This change is nonconservative as aspartic acid has a higher negative charge and is bulkier compared to glycine. This mutation cosegregated with the disease in an affected male and two myopic at-risk carrier females.

DISCUSSION

RP3 is the most prevalent form of XLRP accounting for 70% of cases. Mutations in exons 1 through 19 of *RPGR* have been identified in only 20% of North American XLRP families (Buraczynska et al. 1997). In a previous study, splice site mutations were detected in only 2 of the 11 genetically ascertained *RP3* families (Fujita et al. 1997). These observations suggested that there may be mutations in as yet unidentified exons, regulatory sequences, or even another gene at Xp21.1. Recently Vervoort et al. (2000) identified a new *RPGR* exon, named ORF15, within which mutations were detected in 60% of their XLRP study population (87% from British Isles and Ireland). With the discovery of these mutations, the previous discrepancy between mutations in *RPGR* and frequency of the XLRP sub-type appears to be resolved. It is yet to be determined whether analysis of the North American XLRP population may reveal similar findings for ORF15.

Our samples come from different geographic areas and no mutation has been found to be prevalent. Five of the 27 XLRP families came from Chile; this is the first group of patients from South America where mutation analysis of *RPGR* has been performed. The G436D mutation in exon 11 described here was detected in one of these families. From this, and previous studies, no founder effect seems to be critical in the distribution of the disease. In agreement with previous studies, the new mutations reported in this study are located within the first 2/3 of the gene. Figure 1 summarizes the distribution of the 51 different *RPGR* mutations reported to date (the majority of which are described in The Human Gene Mutation Database; <http://www.uwcm.ac.uk/search/mg/allgenes?>). The mutations have only been detected in exons 1 through 15, which includes the potential GTP binding motif and the highly conserved RCC1-homology region of *RPGR* indicating that this segment is important for the normal function of rod photoreceptors.

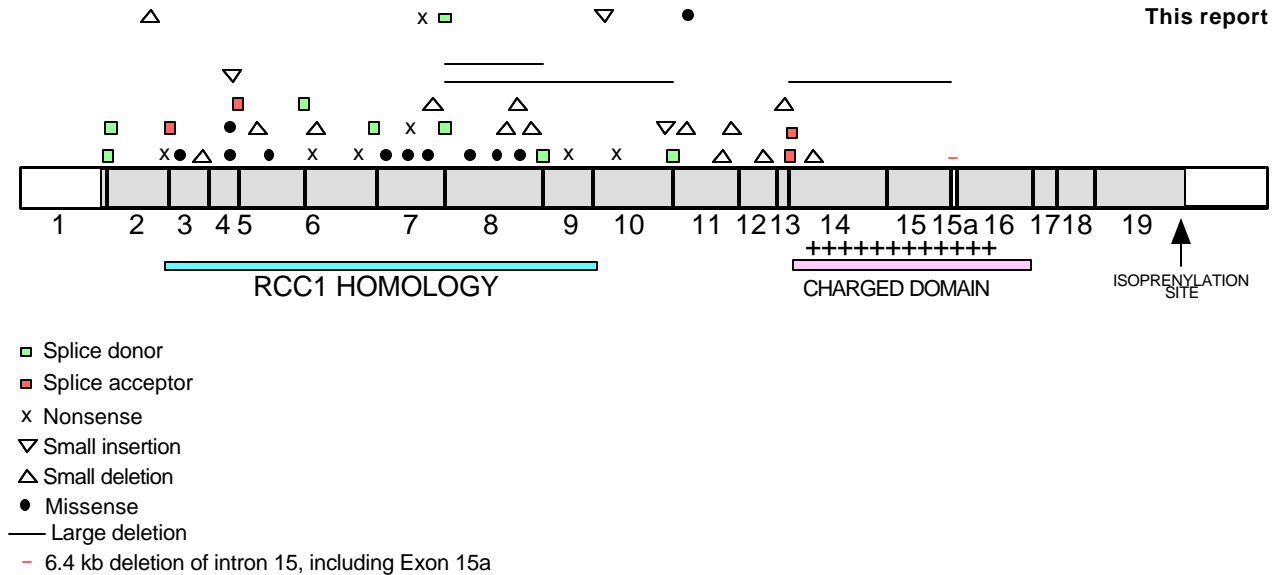


Figure 1. Distribution of *RPGR* mutations reported so far (excluding ORF15)

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