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Protein-Truncation Mutations in the *RP2* Gene in a North American Cohort of Families with X-Linked Retinitis Pigmentosa

To the Editor:

X-linked forms of retinitis pigmentosa (XLRP) are a genetically heterogeneous group of retinal dystrophies that result in relatively severe clinical manifestations (Bird 1975; for a review, see Aldred et al. 1994). The two major XLRP loci, *RP2* (MIM 312600) and *RP3* (MIM 312610), have been mapped to Xp11.32-11.23 and Xp21.1, respectively (for a review see Aldred et al. 1994; Fujita et al. 1996; Fujita and Swaroop 1996; Thiselton et al. 1996). The *RP15* locus (MIM 300029) has been mapped to Xp22.13-22.11 in a single family with retinal degeneration (McGuire et al. 1995), and some evidence exists for a fourth locus, *RP6* (MIM 312612), at Xp21.3 (Musarella et al. 1990; Ott et al. 1990). We recently localized another genetic locus, *RP24* (MIM 300155), at Xq26-27 by using linkage analysis in an XLRP family (Gieser et al. 1998). In addition, the disease in some retinitis pigmentosa (RP) families with apparently X-linked inheritance does not seem to be linked to markers in the region of mapped XLRP loci (Teague et al. 1994; L. Gieser, R. Fujita, and A. Swaroop, unpublished data). It therefore appears that mutations in several genes on the X chromosome may lead to RP.

The first XLRP gene, *RPGR* (retinitis pigmentosa GTPase regulator), was isolated from the *RP3* region (Meindl et al. 1996; Roepman et al. 1996). Genetic analysis has suggested that *RP3* accounts for 70% of XLRP (Ott et al. 1990; Teague et al. 1994; Fujita et al. 1997). However, *RPGR* mutations are detected in only 20% of XLRP (and genetically defined *RP3*) families (Buraczynska et al. 1997; Fujita et al. 1997; M. Guevara-Fujita, S. Fahrner, and A. Swaroop, unpublished data). The *RP2* gene has recently been isolated by a positional cloning strategy (Schwahn et al. 1998) and is predicted to encode a protein of 350 amino acids with homology to cofactor C, which is involved in folding of β-tubulin (Tian et al. 1996). The *RP2* locus is believed to represent 20%–30% of XLRP in Europe (Ott et al. 1990; Teague et al. 1994), but little or no genetic evidence exists for an *RP2* subtype in the XLRP families from North America (Musarella et al. 1990; Ott et al. 1990). Because our haplotype analysis provided suggestive evidence for *RP2* in two North American families (R. Fujita, L. Gieser, S. G. Jacobson, P. A. Sieving, and A. Swaroop, unpublished data), we examined the genomic DNA from our cohort of XLRP patients for causative mutations in the *RP2* gene.

The procedures for clinical ascertainment of patients, obtaining blood samples, and preparation of genomic DNA have been reported elsewhere (Fujita et al. 1997). The families included in the present study showed an apparent X-linked inheritance and no male-to-male transmission. Affected male individuals had a clinical

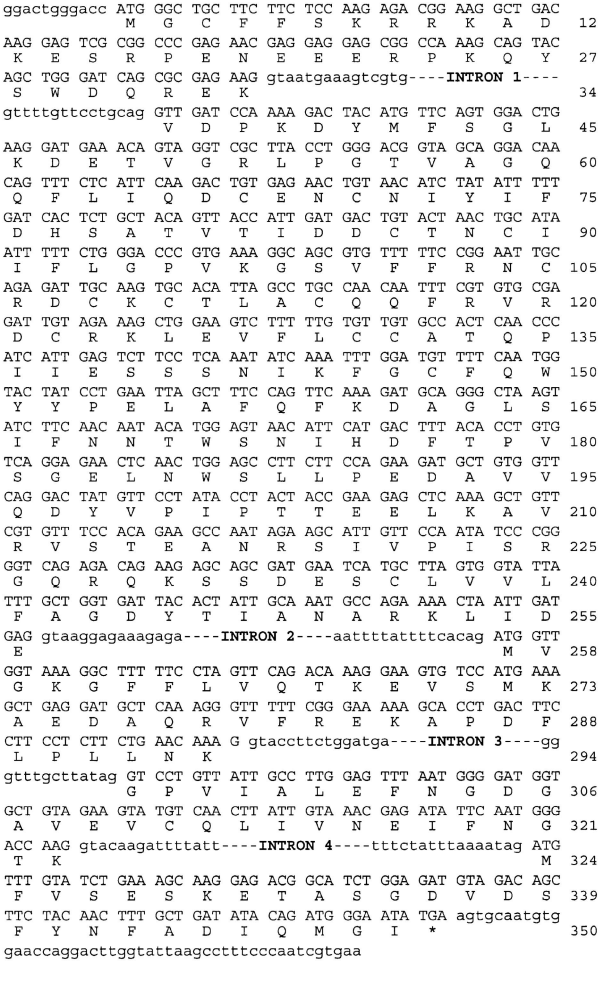

Figure 1 Composite nucleotide sequence showing *RP2* exons, including the coding region, and the exon-intron boundaries. The numbers on the right refer to the amino acid residues of the predicted RP2 protein.

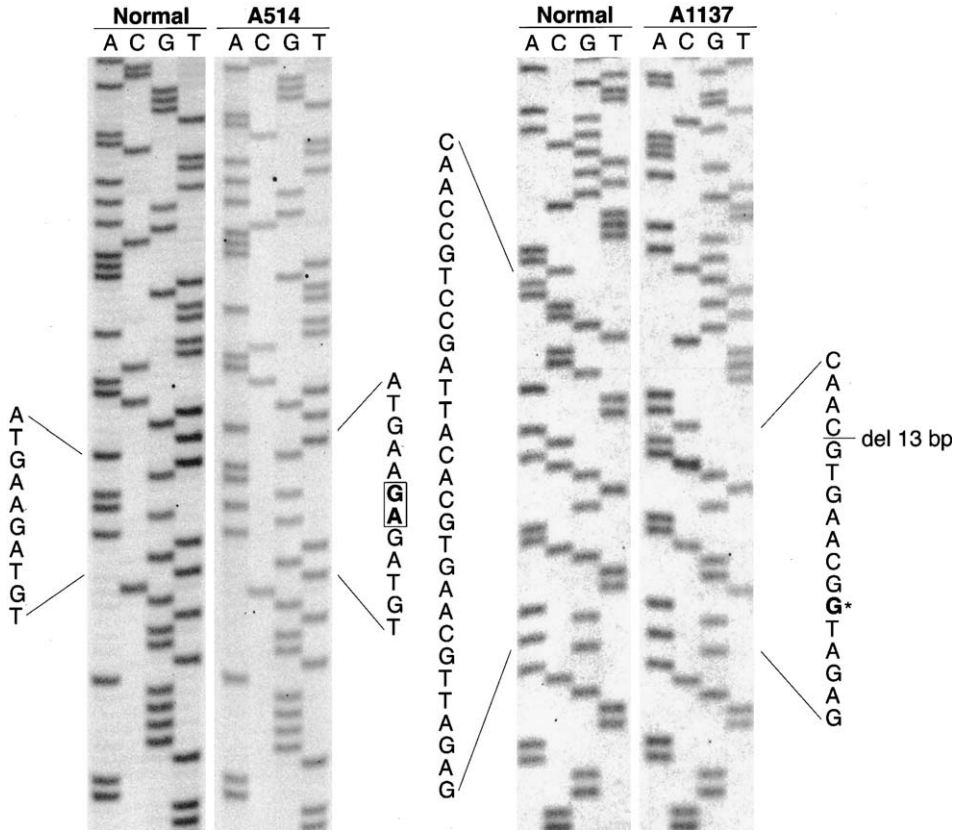

Figure 2 Representative sequencing gels showing two of the *RP2* mutations identified in this report. Sequences in the region of causative mutations are shown. The boxed sequence indicates the 2-bp insertion in patient A514. The location of the 13-bp deletion in patient A1137 is indicated by the horizontal bar. This patient also has a nucleotide substitution, indicated by an asterisk (*).

diagnosis of RP. Initially, one affected male each from 51 XLRP families was included in the *RP2* screening project. This cohort did not include families with a causative *RPGR* mutation or those in which the disease was genetically mapped to the *RP3* locus (see Buraczynska et al. 1997 and Fujita et al. 1997). Oligonucleotide primers flanking each of the five *RP2* exons (Schwahn et al. 1998) were used to amplify products from genomic DNA. PCR products were sequenced with various primers (Schwahn et al. 1998), either directly or after gel purification, by means of the ^{33}P-Thermosequenase cycle-sequencing kit (Amersham Life Science). The composite nucleotide sequence of the *RP2* exons and at the exon-intron boundaries is shown in figure 1. The derived sequence of RP2 polypeptide was identical to that reported elsewhere (Schwahn et al. 1998).

The complete sequencing of *RP2* exons and their corresponding exon-intron junction regions in 51 North American XLRP patients revealed sequence changes in five individuals (fig. 2 and table 1). All of the alterations were identified in the coding region: a 2-bp insertion in exon 1, a 13-bp deletion in exon 2, a nonsense mutation in exon 2, a 7-bp insertion in exon 2, and a 2-bp insertion in exon 4. Except for the C→T change at nucleotide 358 (arginine codon 120 in exon 2), resulting in a nonsense codon, the remaining four changes are deletions or insertions that would cause a frameshift. Therefore, all changes are predicted to result in a truncated RP2 protein. One of the patients (A1137) has an additional sequence alteration (T→G at nucleotide 322, leading to a Cys108Gly change); however, because this individual also has a 13-bp deletion nearby, we did not determine whether the T→G alteration may represent a disease-causing substitution. Each sequence change segregated in complete concordance with the disease in the respective family members that were available for the study (table 1). We suggest, on the basis of the nature of mutations and their cosegregation in respective families, that these sequence changes are causative *RP2* mutations.

This is the first report demonstrating the presence of the *RP2* subtype in North American families with XLRP. In addition to reporting five novel *RP2* mutations, our study addresses several significant issues:

1. The *RP2* mutations that we identified in our North

Table 1

***RP2* Mutations in Patients with X-Linked Retinitis Pigmentosa**

Patient Number	Exon	Nucleotide Sequence Change	Effect of Mutation	Meioses Examined
A2240	1	77/78insCA	Frameshift, 305 amino acids missing	8
A1137	2	T→G at 322 and del 330-342	Cys108Gly and a frameshift, 200 amino acids missing	1
A1135	2	C→T at 358	Arg120Stop, 230 amino acids missing	4
A512	2	483/484insGGGCTAA	Frameshift, 176 amino acids missing	2
A514	4	925/926insAG	Frameshift, 35 amino acids missing	3

NOTE.—Nucleotide positions are indicated according to the *RP2* coding sequence (National Center for Biotechnology Information accession number AJ007590; Schwahn et al. 1998).

American cohort of XLRP families are different from the seven reported in European families (Schwahn et al. 1998), suggesting a high rate of new mutations and a lack of founder effect. Similar observations have been made for *RPGR* mutations in XLRP-RP3 families (Buraczynska et al. 1997).

2. All five mutations reported here are predicted to result in a truncated RP2 protein. Except for Arg118His, the other six mutations identified by Schwahn et al. (1998) would also result in a shorter, or no, RP2 protein. We therefore suggest that the clinical phenotype in most if not all affected XLRP-RP2 families is due to the loss of RP2 function.

3. Our results suggest that it should be possible to identify a majority of *RP2* mutations in XLRP families by a protein-truncation test. Because RP2 protein is widely expressed, a relatively inexpensive diagnostic assay based on immunoblot analysis with RP2-specific antibody (when available) can also be developed. It should be noted that a protein-based diagnostic test has been established for choroideremia, another X-linked retinal dystrophy (MacDonald et al. 1998). Such a test, however, would be hard to develop for RPGR because of the diverse nature of mutations spanning a larger region of protein (Buraczynska et al. 1997) and multiple mRNA and protein isoforms (Yan et al. 1998).

4. Most of the mutations (Schwahn et al. 1998; present article) are detected in exon 2, which can be amplified as a 799-bp product. Additional mutations are present in two small exons—1 and 4. Of interest, no mutation has so far been detected in exon 3 or 5. This clustering of mutations might have significant implications for functional analysis of the RP2 protein and for prenatal and presymptomatic diagnosis.

5. Thus far it appears that screening of both *RPGR* and *RP2* genes leads to identification of disease-causing mutations in fewer than half of XLRP families. The five reported *RP2* mutations were identified by direct sequencing of coding region and exon-intron boundaries. Analysis of the *RP2* promoter region and/or the *RP2* genomic DNA by Southern blotting might reveal additional causative mutations.

Although much of the genetic and phenotypic complexities of XLRP have yet to be resolved, the cloning of *RPGR* and *RP2* genes represents a milestone in RP research. Identification of mutations in these two genes in many XLRP families provides renewed hope for more-precise diagnosis and better genetic counseling for this devastating disease.

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Electronic-Database Information

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

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for *RP2* [MIM 312600], *RP3* [MIM 312610], *RP6* [MIM 312612], *RP15* [MIM 300029], and *RP24* [MIM 300155])

National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/(for RP2 sequence, accession number AJ007590)

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A Fifth Locus for Bardet-Biedl Syndrome Maps to Chromosome 2q31

To the Editor:

Bardet-Biedl syndrome (BBS) is a rare autosomal recessive disorder with major clinical manifestations of retinal dystrophy, obesity, dysmorphic extremities, hypogenitalism, and renal structural and functional abnormalities. It is distinguished from Laurence-Moon syndrome (MIM 245800), Biemond syndrome II (MIM 210350), and Alstrom syndrome (MIM 203800) by the absence of paraplegia, iris coloboma, and perceptive deafness, respectively. Four genetic loci for BBS have been mapped to distinct chromosomes, but the finding, in three recent population surveys, of several unlinked families with