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Report

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

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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 vision loss before the 4th decade. Previously, the *RP15* locus was assigned to Xp22, by linkage analysis of a single pedigree with "X-linked dominant cone-rod degeneration." After clinical reevaluation of a female in this pedigree identified her as affected, we remapped the disease to a 19.5-cM interval (DXS1219–DXS993) at Xp11.4-p21.1. This new interval overlapped both *RP3* (*RPGR*) and *COD1*. Sequencing of the previously published exons of *RPGR* revealed no mutations, but a de novo insertion was detected in the new *RPGR* exon, ORF15. The identification of an *RPGR* mutation in a family with a severe form of cone and rod degeneration suggests that *RPGR* mutations may encompass a broader phenotypic spectrum than has previously been recognized in "typical" retinitis pigmentosa.

The X-linked forms of retinitis pigmentosa (XLRP [MIM 268000]) are among the most severe because of their early onset, often leading to significant vision loss before the fourth decade. Six XLRP loci have been localized by linkage: *RP2* (MIM 312600), *RP3* (MIM 312610), *RP6* (MIM 312612), *RP15* (MIM 300029), *RP23*, and *RP24* (MIM 300155). The two major loci, *RP2* and *RP3,* are clinically indistinguishable and map ~20 cM apart on Xp21. *RP3* accounts for 70% of XLRP (Ott et al. 1990). The *RPGR* (retinitis pigmentosa GTPase regulator) gene isolated from the *RP3* region is mutated in 20% of families with XLRP, including those in which there is evidence of mapping to the *RP3* genetic interval (Buraczynska et al. 1997; Fujita et al. 1997). This discrepancy between predicted and identified mutations in XLRP has recently been resolved by the discovery of a mutational hotspot in a new *RPGR* exon, ORF15 (Vervoort et al. 2000).

The *RP15* locus had been mapped to Xp22.13-p22.11 by linkage analysis of a pedigree described as having dominant cone-rod degeneration (McGuire et al. 1995) although there has been some controversy regarding nomenclature (Daiger et al. 1996; Inglehearn and Hardcastle 1996). Both affected males and "carrier" females presented an early cone involvement in disease. This is different from the typical rod-predominant manifestation of XLRP (Bird 1975; Fishman et al. 1988). *RP15* was localized to an 8.4-cM interval, flanked distally by DXS1229 and proximally by DXS1048. To further delineate the *RP15* interval, for positional cloning, additional markers were analyzed, and new family members were recruited. Recently, it has been discovered that individual IV:4 (fig. 1), originally described as an unaffected female (McGuire et al. 1995), now has a son (V:2) affected with retinitis pigmentosa (RP). In addition, a more recent ophthalmic examination of IV:4 (at age 31 years) has revealed fundus changes (diffuse atrophy, minimal pigment deposits, and attenuation of retinal

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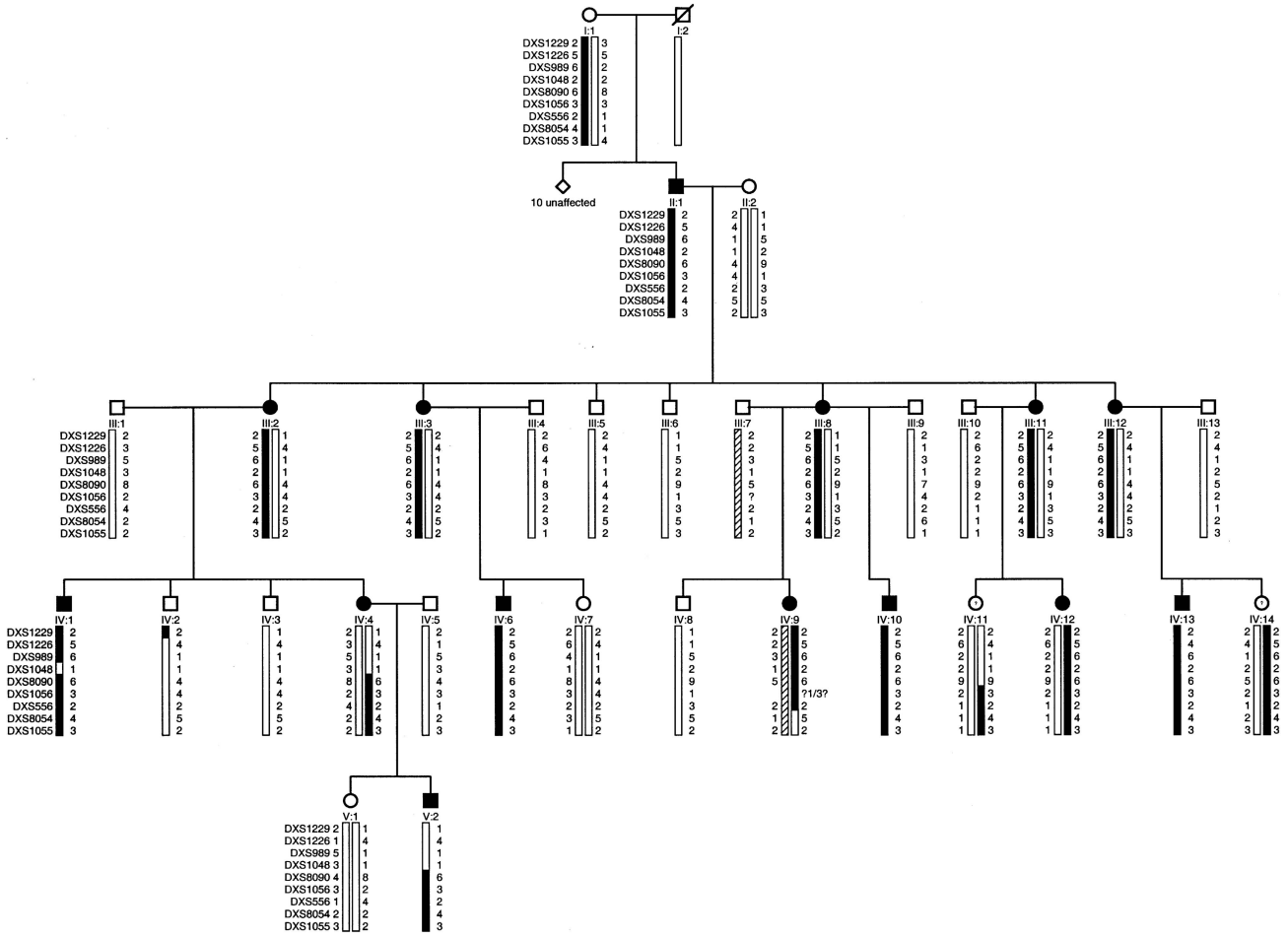

Figure 1 Haplotype analysis of pedigree. Affected individuals are denoted by blackened symbols. Individual I:1 is 92 years old and clinically normal. The clinical status of individuals IV:11 and IV:14 (12 and 2 years old, respectively) is unknown. Individual IV:4 originally had been described as clinically normal (McGuire et al. 1995), but, on more recent ophthalmic examination, has been determined as being affected. The blackened bars represent the disease haplotype segregating in this family; the hatched bar represents the haplotype of III:7, inferred on the basis of the daughter, IV:9.

vessels) and abnormal results on electroretinography (photopic 50% of normal, scotopic 60% of normal) that are consistent with the disease expressed by carrier females in this family. IV:4 has the normal haplotype for the entire *RP15* interval, indicating that the map position for the disease locus in this family had been incorrectly assigned. Haplotype analysis with the X-chromosome microsatellite markers remapped the disease locus distal to DXS8054 and proximal to DXS1048 (fig. 1). Further haplotype analysis of two affected female recombinants—IV:4 and IV:9—further delineated the critical region, as being a 19.5-cM interval flanked proximally by DXS993 and distally by DXS1219 (fig. 2). This newly defined interval overlaps with both *RP3* (*RPGR*) and *COD1* (MIM 304020 [Seymour et al. 1998]). Direct sequencing of the published *RPGR* exons (Meindl et al. 1996; Roepman et al. 1996) in individuals IV:1 (affected male) and IV:2 (normal male) did not reveal any causative mutation. However, analysis of the novel *RPGR* exon ORF15 (Vervoort et al. 2000) identified a single-nucleotide insertion in affected male IV:1 (fig. 3). This mutation results in a frameshift and is predicted to result in premature truncation of the RPGR protein. Interestingly, individual I:1, who has no RP-related visual problems, had 10 unaffected children and 1 affected child (II:1). The mutation was not detected in I:1 but was detected in II:1 and, in subsequent generations, cosegregates with the disease in this pedigree, indicating that it had been a de novo mutation. ORF15, because of the composition and nature of its sequence, is predicted to have a high mutation rate (Vervoort et al. 2000). The identification of a mutation in the *RPGR* gene in this family described as having a severe form of cone and rod degeneration, the cone-degeneration phenotype observed in the

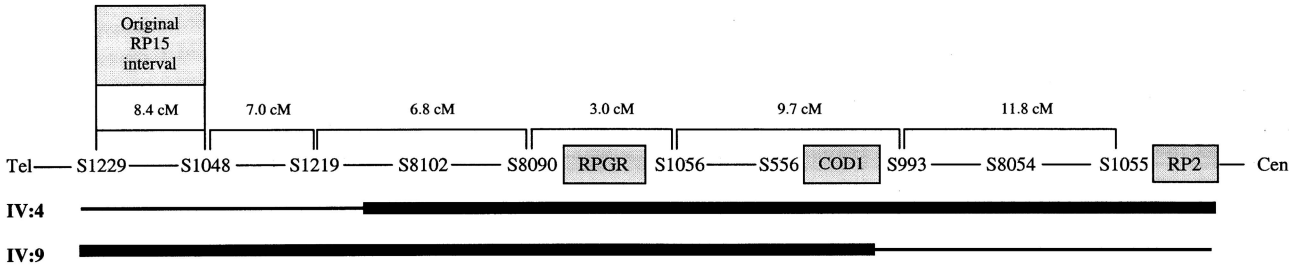

Figure 2 Map of critical recombinants defining the disease interval. The microsatellite markers used to define the interval, as well as their genetic distances, are shown at the top. Relevant disease loci in the region are shown in boxes, relative to the markers. The thickened black lines indicate the disease haplotype in individuals IV:4 and IV:9 (affected females). The disease interval maps between DXS1219 and DXS993, overlapping both *RPGR* (*RP3*) and *COD1*.

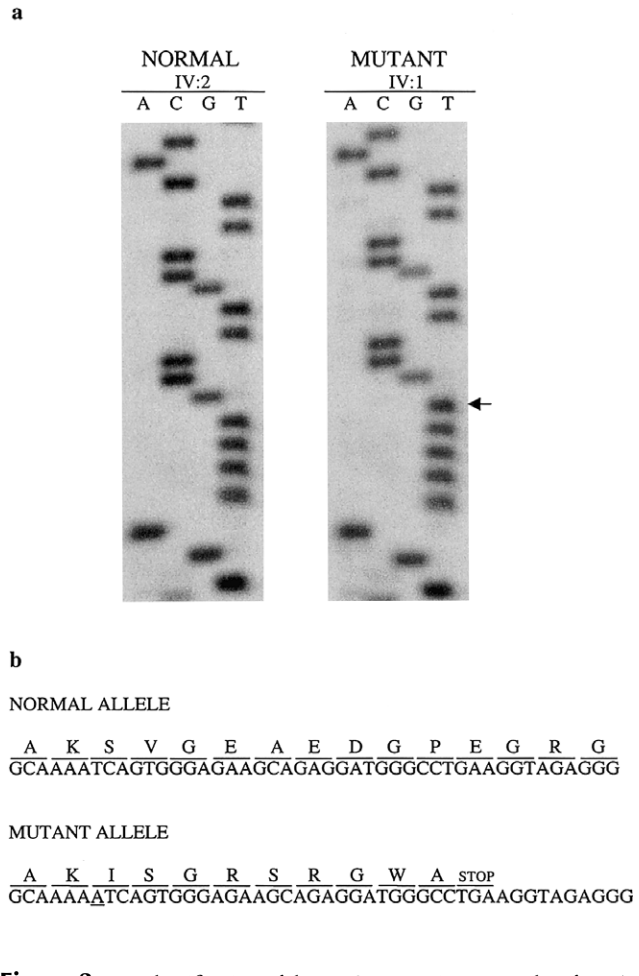

Figure 3 Identification of the *RPGR* mutation. *a*, results of DNA-sequence autoradiograms of IV:2 and IV:1, showing partial sequence from a reverse primer in ORF15. The one-base insertion is indicated by the arrow; this insertion was not detected in 72 unaffected individuals (controls). *b*, Coding sequence and translation comparison of the normal allele and the mutant allele. Partial sequence of ORF15, as well as the predicted translation product, is shown. The single-nucleotide insertion is underlined. The frameshift results in nine novel amino acids and, then, in truncation of the protein product, 501 amino acids premature of the ORF15 stop codon.

Rpgr-knockout mouse (Hong et al. 2000), and the broad clinical spectrum observed in families with XLRP (Keith et al. 1991; Souied et al. 1997; Vervoort et al. 2000) suggest that *RPGR* mutations may encompass a broader phenotypic spectrum than previously has been recognized in "typical" RP.

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Electronic-Database Information

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

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for COD1 [MIM 304020], RP2 [MIM 312600], RP3 [MIM 312610], RP6 [MIM 312612], RP15 [MIM 300029], RP24 [MIM 300155], and XLRP [MIM 268000])

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