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Report

X-Linked Cone-Rod Dystrophy (Locus *COD1*): Identification of Mutations in *RPGR* Exon ORF15

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X-linked cone-rod dystrophy (COD1) is a retinal disease that primarily affects the cone photoreceptors; the disease was originally mapped to a limited region of Xp11.4. We evaluated the three families from our original study with new markers and clinically reassessed all key recombinants; we determined that the critical intervals in families 2 and 3 overlapped the *RP3* locus and that a status change (from affected to probably unaffected) of a key recombinant individual in family 1 also reassigned the disease locus to include *RP3* as well. Mutation analysis of the entire *RPGR* coding region identified two different 2-nucleotide (nt) deletions in ORF15, in family 2 (delAG) and in families 1 and 3 (delGG), both of which result in a frameshift leading to altered amino acid structure and early termination. In addition, an independent individual with X-linked cone-rod dystrophy demonstrated a 1-nt insertion (insA) in ORF15. The presence of three distinct mutations associated with the same disease phenotype provides strong evidence that mutations in *RPGR* exon ORF15 are responsible for COD1. Genetic heterogeneity was observed in three other families, including the identification of an in-frame 12-nt deletion polymorphism in ORF15 that did not segregate with the disease in one of these families.

X-linked cone-rod dystrophy (COD1 [MIM 304020]) is a rare, progressive visual disease primarily affecting the cone photoreceptors. Affected individuals (essentially all of whom are males) present with decreased visual acuity, myopia, photophobia, abnormal color vision, full peripheral visual fields, decreased photopic electroretinographic responses, and granularity of the macular retinal pigment epithelium (Pinckers and Timmerman 1981; Pinckers and Deutman 1987; Jacobson et al. 1989). The degree of rod-photoreceptor involvement can be variable, with degeneration increasing as the disease progresses. Although penetrance appears to be nearly 100%, there is variable expressivity with respect to age at onset, severity of symptoms, and findings (Hong et al. 1994).

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X-linked retinitis pigmentosa (XLRP [MIM 268000]) is a severe form of inherited retinal degeneration that primarily affects the rod photoreceptors (Bird 1975; Fishman et al. 1988). It typically causes an early-onset night blindness and loss of peripheral vision, often causing the patients to become legally blind by the age of 30–40 years. Of the five current distinct XLRP loci, the two major ones are for retinitis pigmentosa type 2 (*RP2* [MIM312600]) and type 3 (*RP3* [MIM 312610]), which were mapped to Xp11.3 (Thiselton et al. 1996; Schwahn et al. 1998) and Xp21.1 (Meindl et al. 1996; Roepman et al. 1996), respectively (see the Web site of RetNet). Although *RP3* accounts for >70% of XLRP (Ott et al. 1990), the original retinitis pigmentosa GTPase regulator gene (*RPGR*), with 19 exons (GenBank accession numbers U57629 and NM_000328) isolated from the *RP3* region, was found to be mutated in only 10%–27% of families with XLRP (Meindl et al. 1996; Roepman et al. 1996; Buraczynska et al. 1997; Zito et al. 2000). Recently, this discrepancy was resolved by the discovery of a mutational hotspot in a new 3′ terminal *RPGR* exon, called "ORF15" (GenBank accession number AF286472), which was found to

Table 1

Primers and Conditions for PCR Amplification of *RPGR* Exon ORF15 Coding Sequence

Fragment	Length (bp)	Annealing Temperature (°C)	Primer (5′→3′)	
			Forward	Reverse
1	348	56	AGGAAGGAGCAGAGGATTCA	CCCTCTTCTTCCATTCTTCC
2	444	56	GGGGAGAAAGACAAGGGTAG	TCCTTTCCCCTCCTCTACTT
3	982	55	GGAAGAAGGAGACCAAGGAG	CCCATTTCCCTGTGTGTTAG
4	414	56	GCAGGATGGAGAGGAGTACA	GAGAGAGGCCAAAATTTACCA

Note.—The first 152 nts of 5′ end sequence of ORF15 correspond to the original *RPGR* exon 15, and the previously described primers for exon 15 were used to amplify this part of the gene (Meindl et al. 1996). The primers in this table produce four overlapping fragments covering the remaining coding region of ORF15.

be mutated in 60% of patients with XLRP; this suggests that mutations in *RPGR* are the only cause of *RP3*-type XLRP (Vervoort et al. 2000). The *RP15* locus (MIM 300029), which was previously assigned to Xp22 by linkage analysis of a single pedigree with X-linked dominant cone-rod degeneration (McGuire et al. 1995), was recently remapped to Xp11.4-p21.1, and a de novo insertion was detected in the *RPGR* exon ORF15 (Mears et al. 2000).

COD1 was originally mapped to a region between Xp11.3 and Xp21.1, encompassing the *RP3* locus (Bartley et al. 1989; Jacobson et al. 1989; Bergen et al. 1993, 1994; Hong et al. 1994; Meire et al. 1994). Subsequent linkage studies, performed by our group through analysis of three families, mapped the disease locus to a limited region of Xp11.4 between the *RP2* and *RP3* loci (DXS993–DXS556), on the basis of the recombinational events in families 1 and 3 and of the screening results for the original *RPGR* transcript (with 19 exons) that were found to be normal in family 2 (Seymour et al. 1998). The detailed clinical descriptions and the pedigrees for these three families have been described elsewhere (Jacobson et al. 1989; Hong et al. 1994; Seymour et al. 1998; Brown et al. 2000). To further narrow the *COD1* interval, additional markers were used, and new dinucleotide-repeat markers were developed by the identification of the dinucleotide repeats in the sequences (through use of the RepeatMasker Web Server) from the genomic clones encompassing the DXS993–DXS556 region and by testing them for a possible polymorphism in selected key individuals. One of the dinucleotide repeats from GenBank clone AC069362 (forward primer, 5′-GCCCTTTGGATGAAAGATCC-3′; reverse primer, 5′-TTGCTTCAGCACACAAGTCA-3′) was found to be polymorphic and informative in both families 1 and 3. BLAST searches revealed that this CA repeat was previously mentioned in GenBank under the accession number Z67588. This new repeat marker (the order is tel-DXS556-DXS8042-[new marker]-DXS574-DXS993-cen) provided the first evidence that the critical intervals for families 1 and 3 were not overlapping in the original DXS993–DXS556 interval. We clinically reassessed all key recombinants and evaluated each family individually for possible heterogeneity. Since the critical intervals in families 2 and 3 were still overlapping with the *RP3* locus (Seymour et al. 1998), *RPGR* (including the subsequently described alternative 3′ terminal exon ORF15) was reconsidered as a candidate and was screened for mutations. In family 1, it was discovered that one of the key recombinants (individual 1: IV:1 [Seymour et al. 1998]), who was initially classified as "affected" because of his symptoms of photophobia and poor color vision, was actually found to have normal central vision with no macular pathology up to his current age of 47 years. He was reclassified as "probably unaffected," and this status change remapped the disease locus in family 1 to include *RP3,* as well.

All 19 original exon fragments, plus ORF15 of the *RPGR* gene from individuals with COD1, were amplified from leukocyte genomic DNA and screened for mutations by direct PCR sequencing, through use of either an ABI377 or ABI3700 sequencer. The intronic primers and corresponding PCR conditions for the 19 original exons have been described elsewhere (Meindl et al. 1996). ORF15 is an alternative 3′ terminal exon that contains exon 15 and extends into part of intron 15 (Vervoort et al. 2000). It has a 1,706-nucleotide (nt) coding sequence (567 amino acids with a repetitive domain rich in glutamic acid residues), and the first 152 nts of 5′ end sequence corresponds to the original *RPGR* exon 15. In addition to the previously published exon 15 primers, we developed and used four primer pairs to produce four overlapping fragments covering the remaining coding region of ORF15 (table 1).

Mutation analysis identified two different 2-nt deletions in ORF15, in family 2 (1339_1340delAG) and in families 1 and 3 (1343_1344delGG), both resulting in a frameshift leading to altered amino acid structure and early termination (fig. 1*a*). These mutations were confirmed by segregation analysis in the original three large families. Additionally, a third, unique frameshift mutation (1564_1565insA) was identified in another individual with X-linked cone-rod dystrophy (fig. 1*b*). None of

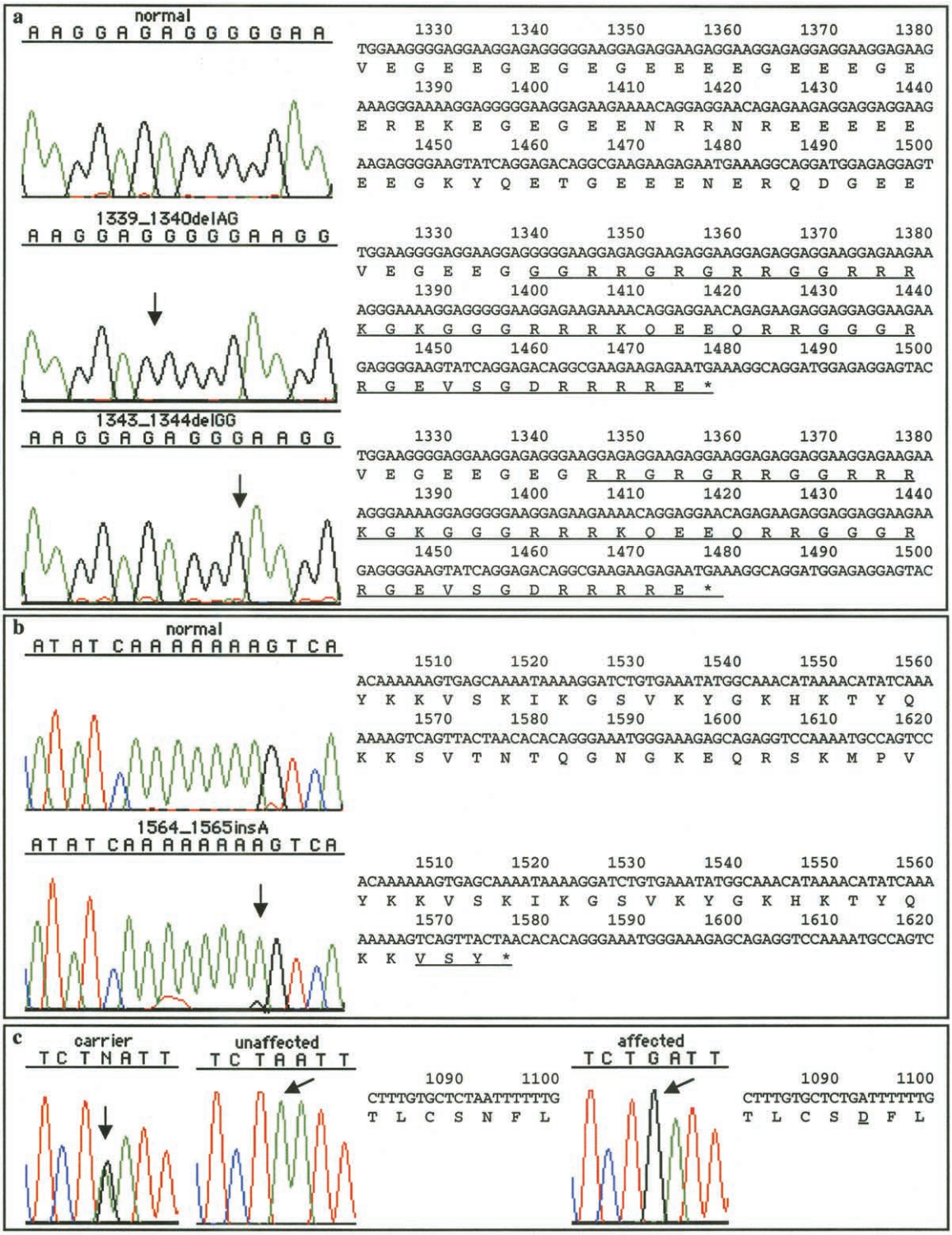

Figure 1 ORF15 mutations and *RPGR* exon 9 sequence alteration, with the corresponding predicted translation products. The locations for sequence alterations are indicated by arrows. The partial sequences of ORF15 (*a* and *b*) and *RPGR* exon 9 (*c*) are shown on the right side of each chromatogram, with the resulting changes in the open reading frame underlined. *a,* The 2-nt deletions identified in the original three families, which cause a frameshift resulting in very similar truncated protein products (46 or 44 novel amino acids, and premature truncation resulting in the loss of 76 amino acids). *b,* The 1-nt insertion identified in another individual with X-linked cone-rod dystrophy, which results in three novel amino acids and in premature truncation resulting in the loss of 43 amino acids. *c,* A nonconservative amino acid change (Asn345Asp) in *RPGR* exon 9 (A1092G), which segregated with the mutation in families 1 and 3 and suggests a shared disease haplotype.

these COD1-related frameshift sequence alterations was detected in 100 control X chromosomes from unaffected subjects.

A nonconservative amino acid change (Asn345Asp) in *RPGR* exon 9 (A1092G) also segregated with the mutation in families 1 and 3 (fig. 1*c*). These results are consistent with the shared disease haplotype that we observed among families 1 and 3 during the linkage analysis (Seymour et al. 1998). The affected males from families 1 and 3 share the same haplotype for *RP3*, [*RPGR* exon 9 (A1092G); ORF15 (1343_1344delGG)]-DXS1068-DXS8025-DXS1058-DXS977-DXS556-DXS8042, corresponding to a region that is not shared by recombinant unaffected individuals. These results suggest that it is highly likely that these two families are related. The same *RPGR* exon 9 missense change was previously reported in one patient with XLRP and was not detected in 150 control X chromosomes (Sharon et al. 2000). However, Sharon et al. (2000) considered this change to be a nonpathogenic variant, since the patient's affected brother did not carry this sequence alteration. This missense change was detected in 2 of 100 control X chromosomes in the unaffected population we screened, which supports the previous consideration that it is a rare sequence variant.

We reconfirmed the existence of genetic heterogeneity for X-liked cone-rod dystrophy (Bergen and Pinckers 1997) on the basis of linkage data and the lack of *RPGR*-ORF15 mutations in three other families, including the observance of an in-frame 12-nt deletion polymorphism that did not segregate with the disease in one of these families. This same polymorphism (1307_1318del12) was also observed in the individual COD1 sample for which we have found the third mutation (1564_1565insA) that prematurely terminates the protein. This polymorphism was detected in 6 of 100 control X chromosomes from unaffected subjects.

Our results indicate that mutations in the *RPGR* exon ORF15 are the primary cause of COD1, on the basis of the three independent mutations identified in the set of families considered here (two of these mutations segregated perfectly with the disease in the three large pedigrees of five to six generations) that give rise to similar C-terminal truncations in the *RPGR*-ORF15 protein. In addition to the already established role of *RPGR* in XLRP (a disease that predominantly affects the rod photoreceptors), the mutations that we identified in the same gene appear to primarily affect the cone photoreceptors and to cause a cone dystrophy phenotype. To the best of our knowledge, these results represent the first definitive evidence that mutations in *RPGR* are associated with COD1. All three COD1 mutations that we identified were in the last 369-nt 3′-end coding sequence of ORF15. Interestingly, only one XLRP mutation has been reported in this region (1344delG [Vervoort et al. 2000]), and the remaining 31 published ORF15 mutations were detected within the preceding 5′ region (Mears et al. 2000; Vervoort et al. 2000; Yokoyama et al. 2001). Affected males in the *RP15* family (with the 173_174insA mutation [Mears et al. 2000]) were reported to have early cone involvement, and the diagnosis for patient 55 (with the 689_692del4 mutation) from Vervoort's series (Vervoort et al. 2000) was "probable" X-linked cone dystrophy. These patients may represent intermediate phenotypes within the broad spectrum (i.e., between typical XLRP and typical COD1) of retinal disease caused by *RPGR* mutations. Further studies will be necessary to determine the role of the *RPGR* exon ORF15 in both rod and cone photoreceptor functions, as well as its impact on the disease phenotype.

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Electronic-Database Information

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

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for *RPGR* [accession numbers U57629 and NM_000328] and ORF15 [accession number AF286472])

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

RepeatMasker Web Server, http://ftp.genome.washington.edu/cgi-bin/RepeatMasker

RetNet, http://www.sph.uth.tmc.edu/Retnet/

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