Mutations in the RPGR gene cause X-linked cone dystrophy

Zhenglin Yang1, Neal S. Peachey1,2,3, Darius M. Moshfeghi1, Sukanya Thirumalaichary1, Lou Chorich4, Yin Y. Shugart5, Keke Fan1 and Kang Zhang1,*

1Cole Eye Institute, I-31, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195, USA, 2Cleveland VA Medical Center and 3Department of Neurosciences, Case Western Reserve University, Cleveland, OH 44106, USA, 4William H. Havener Eye Center, Ohio State University, Columbus, OH 43210, USA and 5Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD 21205, USA

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X-linked cone dystrophy is a type of hereditary retinal degeneration characterized by a progressive dysfunction of the day vision or photopic (cone) system with preservation of night vision or scotopic (rod) function. The disease presents with a triad of photophobia, loss of color vision and reduced central vision. This phenotype is distinct from retinitis pigmentosa (RP) in which there are prominent night and peripheral vision disturbances. X-linked cone dystrophy is a genetically heterogeneous disorder, with linkage to loci on Xp11.4–Xp21.1 (COD1, OMIM 304020) and Xq27 (COD2, OMIM 303800). COD1 maps to a region that harbors the RPGR gene, mutations in which account for >70% of patients with X-linked RP. The majority of these mutations reside in one purine-rich exon, ORF15, encoding 567 amino acids with a repetitive domain rich in glutamic acid residues. We mapped two families with X-linked cone dystrophy to the COD1 locus and identified two distinct mutations in ORF15 in the RPGR gene (ORF15+1343_1344delGG and ORF15+694_708del15) leading to a frame-shift and premature termination of translation in one case and a deletion of five amino acids in another. Consistent with expression of RPGR in rods and cones, our results show that mutations in RPGR, in addition to X-linked RP, can also cause cone-specific degeneration.

INTRODUCTION

The cone dystrophies are a heterogeneous group of hereditary retinal degenerations characterized by progressive dysfunction of the photopic (cone-mediated) system, presenting with photophobia, loss of color vision and reduced central visual acuity. This phenotype is in contrast to that of retinitis pigmentosa (RP), which is characterized by abnormalities in scotopic (rod-mediated) functions, manifesting as night blindness and a decrease of peripheral vision. Cone dystrophy is genetically heterogeneous and may present as an autosomal dominant or X-linked recessive trait. Autosomal loci have been localized to 6p21.1 (COD3, OMIM 602093) (1), 6q25–q26 (OMIM 180020) and 17p12–13 (OMIM 601251) (2,3). In the case of COD3, mutations have been identified in the GUCA1A gene encoding GCAP1 (1,4,5), a Ca2+-binding protein involved in regulation of rod and cone phototransduction (6). X-linked cone dystrophy maps to Xp11.4–21.1 (COD1, OMIM 304020) (7–9) and Xq27 (COD2, OMIM 303800) (10).

X-linked RP is the most severe form of RP. Patients tend to present with night blindness and constiction of visual field during the third and fourth decades of life (11). In comparison, visual acuity and color vision can be normal in RP patients until advanced stages of the disease. X-linked RP is genetically heterogeneous, with loci localized to Xp11.2 (RP2), Xp21.1 (RP3) and Xp21.2–21.3 (RP6), Xp22 (RP23) and Xq26–27 (RP24) (http://www.sph.uth.tmc.edu/RetNet/disease.htm). Genes for RP2 and RP3 have been cloned (12–15). The RPGR (RP3) gene encodes a protein with homology to RCC1 (regulator of chromatin condensation-1), a guanine nucleotide exchange factor for the small GTPase Ran, a protein involved in nuclear trafficking. RPGR interacts with a protein termed RPGR-interacting protein (RPGRIP) (16–18). In addition, cones, as well as rods, degenerate in mice null for the RPGR gene (19). The RPGR gene was mutated in >70% of patients with X-linked RP and the majority of mutations resides in one purine-rich exon, ORF15, encoding 567 amino acids, with a repetitive domain of low sequence complexity with high glutamic acid and glycine content (15). ORF15 is preferentially expressed in mouse, bovine and human retina and is conserved in the pufferfish (Fugu rubripes), mouse, bovine and human (15). All mutations of ORF15 in RP3 patients were small nucleotide deletions or nonsense mutations in the 5′-end or the central portion of ORF15, resulting in premature truncation of translation (15,20,21). The high frequency of mutations within ORF15 compared with other parts of the same RPGR transcript suggested that it is a mutation hotspot (15).

*To whom correspondence should be addressed at: Moran Eye Center, Department of Ophthalmology and Visual Science, University of Utah, 50 North Medical Drive, Salt Lake City, UT 84132, USA. Tel: +1 801 585 2019; Fax: +1 801 585 3501; Email: zhangk@ccf.org

Present addresses:
Zhenglin Yang, Sukanya Thirumalaichary, Keke Fan and Kang Zhang, Moran Eye Center, Department of Ophthalmology and Visual Science, University of Utah, 50 North Medical Drive, Salt Lake City, UT 84132, USA.
Mutations in \( RPGR \) have been identified mainly in patients with the diagnosis of RP. We mapped two families with X-linked cone dystrophy to the \( \text{COD1} \) locus and identified two distinct mutations in ORF15 in the \( RPGR \) gene (ORF+1343_1344delGG and ORF+694_708del15). The observation that mutations in \( RPGR \) can cause cone-specific degeneration indicates that in addition to a well established role for rod photoreceptors, \( RPGR \) also plays an important role in cone photoreceptors.

**RESULTS**

**Clinical evaluations**

Clinical evaluations on two kindreds revealed an X-linked pattern of inheritance (Fig. 1). On the basis of the presence of photophobia, decreased visual acuity, color vision and macular abnormality, two of nine male individuals at risk for inheriting the disease in family A and two of four male individuals in family B were found to be affected. Decreased visual acuity and color vision appeared at ~45 years of age, followed by progressive macular atrophy (Fig. 2A–C). Figure 2D presents electroretinograms (ERGs) recorded under conditions that allow rod- and cone-mediated function to be compared. Rod-mediated responses recorded under dark-adapted conditions were clearly present in both patients from whom ERGs were obtained (Fig. 2D, upper two rows). The amplitude reduction observed in patient III:3 from family A may be attributed at least in part to the high myopia present in this individual (22). Consistent with this interpretation, dark adaptation recovery measured in this individual using a Goldmann–Weekers dark adaptometer proceeded with normal kinetics and achieved a final threshold that fell within the normal range (data not shown). In comparison, cone-mediated responses were markedly reduced in amplitude in both affected patients tested by ERG (Fig. 2D, lower two rows). Overall, these ERG results indicate a generalized loss of cone function, with preservation of rod function (Fig. 2D). Goldmann visual fields obtained from individual III:3 in family A and III:1 in family B showed that peripheral visual fields were preserved (data not shown). Taken together, clinical evaluations demonstrated that the disease phenotype in these two study families is that of a cone dystrophy.

**Genetic analysis**

An initial genotype analysis using polymorphic DNA markers DXS9896 and DXS6810 in family A demonstrated positive linkage to the \( \text{COD1} \) locus. A two-point peak LOD score of 1.19 was obtained at \( \theta = 0 \) with marker DXS6810. In affected patients, the inheritance of a disease haplotype was concordant with the disease phenotype. Since \( RPGR \) is located in the proximity of \( \text{COD1} \) and fell within the minimal genetic interval in this family, we performed mutational screening in the \( RPGR \) gene. Single-strand conformational polymorphism (SSCP) analysis followed by direct sequencing revealed a 2 bp deletion (ORF15+1343_1344delGG). This deletion resulted in a frame-shift, loss of a 120 amino acid long fragment at the C-terminus and synthesis of an aberrant peptide from amino acid 448 to 491 followed by a premature stop (Fig. 3). We genotyped and then screened the \( RPGR \) gene in family B with X-linked cone dystrophy. We found a 15 bp deletion in nucleotides 694–708 of ORF15 resulting in elimination of five amino acids from 221 to 225 (Fig. 3B, ORF15+694_708del15). These changes segregated with the disease phenotype in each family, and were not found in 150 normal control subjects.

**DISCUSSION**

In this study we show that mutations in ORF15 of \( RPGR \) result in X-linked cone dystrophy (\( \text{COD1} \)) in two families. Several lines of evidence support the contention that a sequence change of ORF15+1343_1344delGG in \( RPGR \) is a pathogenic mutation. First, this change segregated with the disease phenotype in family A (Fig. 1). Secondly, this 2 bp deletion predicted a severe change, resulting in truncation of a 120 amino acid long fragment at the C-terminus and synthesis of an aberrant peptide from amino acid 448 to 491 followed by a premature stop (Fig. 3A). We genotyped and then screened the \( RPGR \) gene in family B with X-linked cone dystrophy. We found a 15 bp deletion in nucleotides 694–708 of ORF15 resulting in elimination of five amino acids from 221 to 225 (Fig. 3B, ORF15+694_708del15). These changes segregated with the disease phenotype in each family, and were not found in 150 normal control subjects.
nor ORF15+694_708del15 were present in 150 normal control subjects in our population, in addition to being absent in 150 control chromosomes in a previous study (15).

The function of RPGR is unknown. The presence of an RCC1 homology domain in the N-terminal domain of RPGR raises the possibility that RPGR may regulate intracellular transport in photoreceptors through a yet unidentified G protein. RPGR interacts with RPGRIP, a protein specifically expressed in the retina (16–18). Mutations in RPGRIP cause Leber’s congenital amaurosis (23,24).

Two lines of evidence support the notion that RPGR plays a role in cone photoreceptors: (i) RPGR is expressed in cone photoreceptors and (ii) knockout mice lacking the RPGR gene exhibit abnormal transport of cone opsins, in addition to defects in rhodopsin transport (19). In the late stage of retinal development, both rod and cone degeneration occur in RPGR knockout mice. Other support for primary cone disease as an important manifestation of RPGR mutations is provided by linkage of this site to X-linked dominant cone-rod degeneration (25) and by functional analysis of RP3 heterozygotes (26).
It is not clear why some *RPGR* mutations cause cone dystrophy while others cause RP. Mutations causing *COD1* are either a truncating mutation located in the C-terminus of the *RPGR* protein or an in-frame five amino acid deletion in *ORF15*. In contrast, all *RP3* mutations are truncating mutations that are clustered in the N-terminus or central portion of the *ORF15*, or in other exons that are located 5' to *ORF15*. While it seems reasonable to hypothesize that *RPGR* mutations restricted to the C-terminus may be associated with cone dystrophy whereas more severe terminating mutations are associated with RP, the mechanism(s) underlying this clinical heterogeneity remains to be elucidated. It is possible that different features of *RPGR* activity are required in rod and cone photoreceptors, such that cone photoreceptors are more sensitive to certain *RPGR* mutations than rod photoreceptors and vice versa. Alternatively, the C-terminus of *RPGR* may interact with a cone photoreceptor-specific component to confer cone specificity. Mutations in a single gene causing different phenotypes have been well documented in retinal diseases. For example, null mutations in the *ABCA4* gene cause autosomal recessive RP, whereas missense mutations cause recessive Stargardt macular dystrophy (27,28). In another example, distinct mutations in the *RDS* gene are linked to dominant RP, dominant macular dystrophy, digenic RP (with ROM1) and dominant adult vitelliform macular dystrophy (29–31).

In the future, it will be important to develop a more complete understanding of the role(s) that *RPGR* plays in rod and cone photoreceptors. This information will provide the foundation for understanding the different effects that *RPGR* mutations may have on rods, to produce an RP phenotype, or on cones, to generate cone dystrophy.

**MATERIALS AND METHODS**

**Subjects**

Approval from the Institutional Review Board of the Cleveland Clinic Foundation was obtained for this study and informed consent was obtained from all participants.
consent was obtained from all patients. Seventeen individuals, including nine males at risk for inheriting cone dystrophy in the first Caucasian kindred, and eight individuals, including four males at risk for the disease, participated in the study. Both families are of northern European origin, as are the 150 normal control subjects.

Clinical investigations
A visual history was obtained from all patients and best-corrected visual acuities were assessed. Ophthalmoscopic examination was performed on all patients, and fluorescein angiography on three affected patients. Blood samples were obtained by venipuncture.

After initial dark adaptation, ERGs were recorded using a monopolar Burian–Allen contact lens electrode, to strobe flash stimuli presented within an LKC ganzfeld. Reference and ground leads were placed on the forehead and earlobe, respectively. The response was differentially amplified (band-pass: 0.3–500 Hz), averaged and stored using an LKC UTAS E-2000 signal-averaging system. Responses were initially recorded to flashes presented to the dark-adapted eye, using low intensity (−2.0 log cd s/m²) and high intensity (0.5 log cd s/m²) stimuli. A steady rod-desensitizing adapting field (1.5 log cd/m²) was then presented in the ganzfeld bowl. After a 7 min period of light adaptation (32), cone ERGs were recorded to 0.5 log cd s/m² flashes presented at 0.7 Hz and 31 Hz.

Genetic linkage and mutation screening
DNA was extracted from blood samples and genetic linkage assessed using microsatellite markers DXS9896 and DXS6810 tightly linked to the COD1 and RPGR loci using established methods (33). An X-linked recessive mode of inheritance with full penetrance was used for LOD score computation. Disease allele frequency was set at 0.0001. Mutational screening of the 15 exons of the RPGR gene was performed by SSCP analysis followed by direct sequencing of PCR-amplified DNA fragments corresponding to each exon of the gene using established methods (13–15,34). PCR primers were designed according to published sequence (13,14) except for ORF15. The following primers were used to amplified overlapping fragments of ORF15: 5′-CATGGAAAGGTGCAAGTGAGA-3′ and 5′-TCCA-TCTTTGGTTTCTTTGCTCTC-3′ (651 bp product); 5′-GCAGAACACTGGCAAGATGA-3′ and 5′-TCTTCCCTTTCTTCTCCC-3′ (596 bp product); 5′-AGGAAGAGGAAGGGGTAGAGAAGTGAAGAG-3′ and 5′-TTCTTTCCTGTCTCTCTGAT-3′ (743 bp product); 5′-GAAGAGGAGGAAAGGAGAAGGGAG-3′
and 5′-AGTGCCCGTTATATGCAGG-3′ (583 bp product). Direct sequencing was performed with the Taq Dydeoxy Terminator Cycle Sequencing Kit (Beckman-Coulter, Fullerton, CA) according to the manufacturer’s instructions.

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NOTE ADDED IN PROOF
An independent study has recently identified three different frame-shift ORF15 mutations in X-linked cone–rod dystrophy (COD1): one of these mutations is the same as we identified in our family (ORF15+1343_1344delGG) and was detected in two probably related families in that study (35).

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