Am. J. Hum. Genet. 63:1307–1315, 1998

A Range of Clinical Phenotypes Associated with Mutations in *CRX,* a Photoreceptor Transcription-Factor Gene

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Summary

Mutations in the retinal-expressed gene *CRX* (cone-rod homeobox gene) have been associated with dominant cone-rod dystrophy and with de novo Leber congenital amaurosis. However, CRX is a transcription factor for several retinal genes, including the opsins and the gene for interphotoreceptor retinoid binding protein. Because loss of CRX function could alter the expression of a number of other retinal proteins, we screened for mutations in the *CRX* gene in probands with a range of degenerative retinal diseases. Of the 294 unrelated individuals screened, we identified four *CRX* mutations in families with clinical diagnoses of autosomal dominant cone-rod dystrophy, late-onset dominant retinitis pigmentosa, or dominant congenital Leber amaurosis (early-onset retinitis pigmentosa), and we identified four additional benign sequence variants. These findings imply that *CRX* mutations may be associated with a wide range of clinical phenotypes, including congenital retinal dystrophy (Leber) and progressive diseases such as cone-rod dystrophy or retinitis pigmentosa, with a wide range of onset.

Introduction

Inherited retinal diseases are exceptionally heterogeneous, both genetically and phenotypically. A total of 96 genes causing inherited retinal diseases have been mapped to chromosomal sites in man, but less than half have been cloned (RetNet; Daiger et al. 1998). Not only is the diagnosis of some of these diseases difficult because of overlapping phenotypes, but mutations within a single gene may cause very different phenotypes. For example, different mutations in one gene, peripherin/*RDS,* have been associated with several forms of retinal degeneration, such as cone-rod dystrophy, cone degeneration, and retinitis pigmentosa, affecting both the macula and the panretinal structures (Weleber et al. 1993; Wells et al. 1993; Keen et al. 1994; Nakazawa et al. 1994, 1996).

Recently, mutations within the photoreceptor-expressed gene *CRX* (cone-rod homeobox gene; MIM 120970) have been reported to cause dominant cone-rod dystrophy at the CORD2 locus (Freund et al. 1997; Swain et al. 1997); in addition, de novo CRX mutations have been found in isolated cases of Leber congenital amaurosis (Freund et al. 1998). *CRX* belongs to the orthodenticle homeobox (*OTX*) family of homeobox genes, members of which are involved in the development of anterior head structures and other embryonic features (Finkelstein and Boncinelli 1994). The three exons of *CRX* encode a 299–amino acid polypeptide, which shows a high degree of sequence similarity to OTX and OTX-related homeodomain proteins: the *CRX* homeobox sequence has up to 88% identity with the homeobox sequences of other members of the *OTX* gene family. In addition to the homeodomain, *CRX* and the other *OTX*-related genes share two highly conserved peptide sequences, a 13–amino acid WSP motif and a 12–amino acid OTX tail (Furukawa et al. 1997; Swain et al. 1997; Freund et al. 1998). CRX is also expressed in the pineal gland and is a transcription factor for pineal-specific genes (Li et al. 1998).

CRX binds specifically to conserved sequences upstream of several photoreceptor-specific genes, including the opsins, and CRX activates transcription of interphotoreceptor retinoid binding protein, arrestin, and β-phosphodiesterase (Chen et al. 1997; Furukawa et al. 1997). Because CRX appears to affect many retinal genes, mutations within the *CRX* gene may be associated with different retinal-disease phenotypes.

We screened the *CRX* gene in a large cohort of patients with a wide range of clinical diagnoses (table 1). We

Received April 14, 1998; accepted for publication September 12, 1998; electronically published October 16, 1998.

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0002-9297/98/6305-0008$02.00

Table 1

Clinical Diagnoses of Individuals Screened for *CRX* Mutations, in This Study

Clinical Diagnosis	No. of Unrelated Probands Tested
Retinitis pigmentosa, autosomal dominant	164
Retinitis pigmentosa, autosomal recessive	22
Retinitis pigmentosa, isolated	63
Cone-rod dystrophy or degeneration, autosomal dominant	24
Rod-cone dystrophy, autosomal dominant	6
Cone degeneration, autosomal dominant	6
Bardet-Biedl syndrome, autosomal recessive	3
Leber congenital amaurosis, autosomal dominant	1
Leber congenital amaurosis, isolated	1
Chorioretinitis, isolated	2
Usher syndrome, autosomal recessive	1
Optic atrophy, autosomal dominant	1

report a new *CRX* mutation associated with autosomal dominant cone-rod dystrophy in one family and the E80A mutation reported by Freund et al. (1997) in a second, unrelated family with cone-rod dystrophy. The present study also demonstrates association between *CRX* mutations and a family with late-onset, autosomal dominant retinitis pigmentosa or mild, atypical, cone-rod dystrophy and a family with severe congenital cone-rod dystrophy with the clinical description "dominant Leber congenital amaurosis" (Heckenlively 1988). Clinical summaries of the diseases in these families are presented, to emphasize the broad impact of *CRX* mutations.

Subjects, Material, and Methods

Subjects

Subjects were ascertained at one of the following sites: (1) the Anderson Vision Research Center, Retina Foundation of the Southwest, Dallas (108 unrelated probands); (2) the Jules Stein Eye Institute, UCLA School of Medicine, Los Angeles (167 unrelated probands); or (3) the Hermann Eye Center, Department of Ophthalmology, The University of Texas Health Science Center, Houston (19 unrelated probands, including the large cone-rod dystrophy family from Texas). Informed consent was obtained from all subjects. For each case, clinical evaluation was by at least one of the coauthors and, for several cases, by two coauthors jointly. Families in which *CRX* mutations were found are shown in figures 1–4; individuals from whom DNA samples were obtained and tested are indicated by "DNA" to the upper right of the symbol.

DNA Isolation

DNA was isolated from peripheral blood by use of the Puregene DNA extraction kit (Gentra), in accordance with the manufacturer's instructions.

Genotyping

Primer pairs for the chromosome 19 linkage markers were obtained from Research Genetics. The forward-strand primer was end labeled with ^{32}P-ATP and polynucleotide kinase (Promega). Amplified DNA was diluted 2:3 (vol:vol) with a solution of 95% formamide, 20 mM EDTA, 0.05% bromophenyl blue, and 0.05% xylene cyanol, and fragments were separated on 6% denaturing acrylamide gels (Promega).

SSCP Analysis

Genomic DNA samples from patients were screened by SSCP, by use of five sets of primers (table 2). All primers were synthesized by a commercial source (Genosys Biotechnologies). PCR was performed with AmpliTaq polymerase (Perkin Elmer). Products were radiolabeled by incorporation of 1 μCi ^{32}P-dCTP. After an initial denaturation step for 5 min at 95°C, PCR was conducted for 30 cycles, each with a denaturation step of 30 s at 95°C. Annealing was for 30 s at 64°C for fragments 1, 2, 3a, and 3b. Annealing for fragment 3c was for 30 s at 60°C. Extension for all fragments was for 30 s at 72°C, with a final elongation step for 10 min at 72°C. The amplified products for exons 1, 2, and 3a were analyzed as described elsewhere by Freund et al. (1997). In this study, the 3b fragment of Freund et al. (1997) was amplified in two smaller independent and overlapping fragments, 3b and 3c. The amplification products of exon 3c were digested with *Eco*RI (Stratagene), yielding fragments of 209 bp and 132 bp for SSCP analysis.

For SSCP, PCR products were denatured and separated on a 0.6 × MDE gel (FMC Bioproducts), either at room temperature or at 4°C. The gel was prepared in 0.6 × Tris-borate EDTA buffer and was subjected to autoradiography after electrophoresis.

Sequence Analysis

In general, for sequence analysis, the fragment showing an SSCP variant was amplified, by PCR, from the stock DNA for the patient. The fragment then was treated with shrimp alkaline phosphatase (Amersham) and exonuclease I (Amersham), followed by sequencing with the AmpliCycle sequencing kit (Perkin Elmer) and a primer end labeled with ^{33}P-ATP. The PCR sequencing

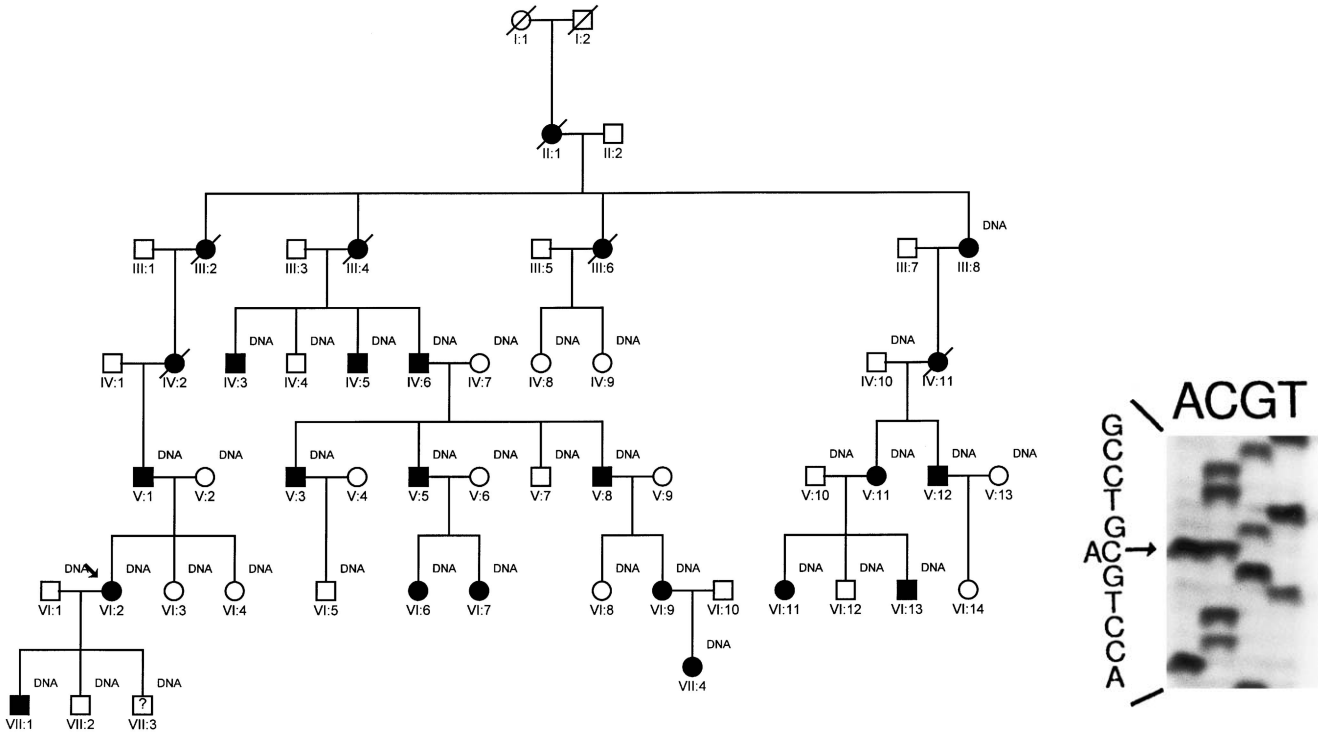

Figure 1 Family UTAD148, cone-rod dystrophy, autosomal dominant. *Left,* Pedigree. Blackened symbols represent affected individuals. A question mark (?) designates an individual of unknown phenotype. "DNA" to the upper right of the symbol indicates that a DNA sample was tested. *Right,* Sequence of exon 2, showing the A→C substitution in one allele (indicated by arrow), at nucleotide 238.

protocol consisted of a 2-min denaturation step at 95°C, followed by 30 cycles of 30 s at 95°C and 30 s at 60°C each. The sequence was separated on 6% Long Ranger (FMC) denaturing acrylamide gels.

For insertion or deletion mutations detected by PCR-product sequencing, a second amplification of the fragment was followed by subcloning with the PCR-Script Amp Cloning kit (Stratagene). The fragment then was sequenced by use of vector-specific primers, by the SeqExpress automated sequencing laboratory of Research Genetics. Exact sizes of deletions or insertions were determined by comparison of the mutated sequence with the wild-type sequence (GenBank cDNA accession number AF024711).

The E80A mutation was assayed in all individuals from family UTAD148, by amplification of *CRX* exon 2 followed by restriction digestion. *CRX* exon 2 was amplified from patient genomic DNA, by PCR using the conditions described above, and was digested with *Hin*fI (Stratagene) for 2 h at 37°C. The enzyme was inactivated with a final 10-min "soak" at 80°C, and digested products were separated on a nondenaturing 2.5% agarose gel stained with ethidium bromide. Fragments of 229 bp and 85 bp were observed for each normal allele, and a fragment of 314 bp was observed for each mutant allele.

Results

Of the 294 probands from unrelated families screened for *CRX* mutation (table 1), we identified four disease-associated mutations in families with inherited retinopathies and an additional four benign variants (table 3). Of the 24 unrelated individuals from families with autosomal dominant cone-rod dystrophy, 2 have *CRX* mutations. Of the 164 families with autosomal dominant retinitis pigmentosa, 1 has a *CRX* mutation. Later analysis of additional members of the retinitis pigmentosa family suggested an alternative diagnosis of late-onset, atypical, cone-rod dystrophy. No *CRX* mutations were observed in samples from pedigrees with isolated retinitis pigmentosa or recessive retinitis pigmentosa. An additional *CRX* mutation was found in a family with autosomal dominant Leber congenital amaurosis. These mutations were not observed in any of the other unrelated individuals screened, in a total of 588 chromosomes.

Family UTAD148, Cone-Rod Dystrophy, Autosomal Dominant

Linkage of cone-rod dystrophy in a large family from Texas, UTAD148 (fig. 1), to the *CORD2* locus was dem-

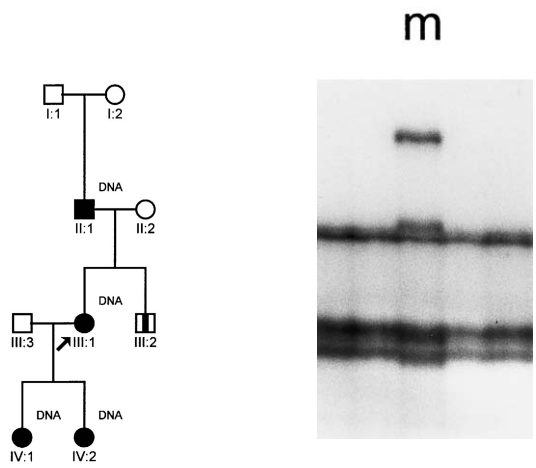

Figure 2 Family RFS014, cone-rod dystrophy, autosomal dominant. *Left,* Pedigree. Blackened symbols represent affected individuals. The symbol with a black bar represents an individual whose affected status was determined by "hearsay." "DNA" to the upper right of the symbol indicates that a DNA sample was tested. *Right,* Results of SSCP analysis of exon 3b, showing aberrantly migrating fragment in the lane designated "m."

onstrated by use of 10 microsatellite markers in the region, with a maximum LOD score of 7.14 at 0% recombination to D19S902 (Sohocki et al. 1997). Five affected members of this family (fig. 1) were shown, by SSCP analysis of *CRX,* to share a variant band in exon 2. Sequencing revealed an A→C transversion at codon 80 (fig. 1), resulting in an amino acid change from glutamic acid to alanine (E80A). Because this sequence change eliminates a *Hin*fI restriction site, PCR amplification of exon 2 followed by restriction with *Hin*fI was used to confirm that the E80A mutation was present in all affected individuals ($n = 18$) and was lacking in all unaffected individuals ($n = 17$) available from this family.

This is the same mutation reported, by Freund et al. (1997), to be associated with cone-rod dystrophy in a Greek family. To determine if the Texas and Greek families share a common ancestor with the mutation or if the mutations in the two families arose independently, haplotype analysis was performed. Two DNA samples from affected members of the Greek family were compared with those from the Texas family, by use of three markers tightly linked to the *CORD2* locus: D19S902, D19S606, and D19S412 (Lawrence Livermore National Laboratory; Sohocki et al. 1997). For each marker, the allele shared by affected members of the Texas family differed from that shared by affected members of the Greek family (data not shown).

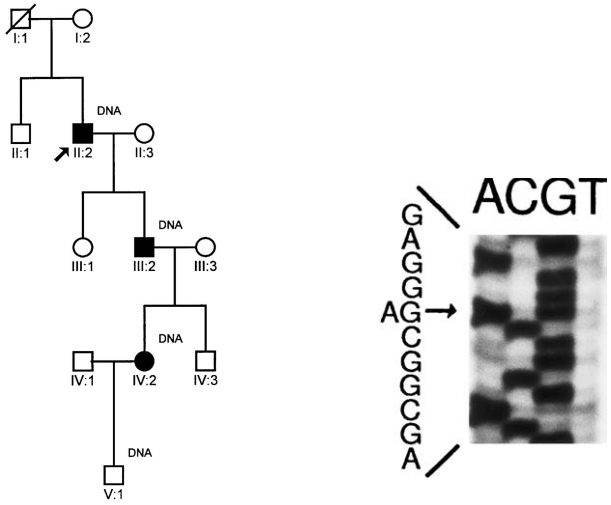

Figure 3 Family RFS087, retinitis pigmentosa, autosomal dominant. *Left,* Pedigree. Blackened symbols represent affected individuals. "DNA" to the upper right of the symbol indicates that a DNA sample was tested. *Right,* Sequence of exon 2, showing the A→G substitution in one allele (indicated by arrow), at nucleotide 121.

Family RFS014, Cone-Rod Dystrophy, Autosomal Dominant

An affected member of this family (fig. 2) was shown, by SSCP analysis, to have a variant band for exon 3b (fig. 2). PCR-product sequencing suggested a 1-bp insertion in codon 196 (Ala196+1 bp). The fragment was amplified and subcloned, and the clones were sequenced, confirming the 1-bp insertion. The mutation was identified in an additional affected family member, by SSCP.

Family RFS087, Retinitis Pigmentosa (Late Onset with Cone-Rod Involvement), Autosomal Dominant

One affected member of this family (fig. 3) was shown to have an SSCP variant in exon 2. Sequencing revealed a G→A transition in codon 41 (fig. 3), resulting in a missense mutation substituting a glutamine for arginine (R41Q). This mutation was confirmed, by SSCP, in two additional affected family members and was absent from an unaffected member (fig 3A). The R41Q mutation was also reported by Swain et al. (1997), for a family with cone-rod dystrophy.

Family RFS900, Leber Congenital Amaurosis (Early-Onset Retinitis Pigmentosa), Autosomal Dominant

Two affected members of this family (fig. 4) were shown, by SSCP analysis, to have a variant in the overlapping segment of exons 3a and 3b (fig. 4). PCR-product sequencing suggested a 12-bp deletion beginning in codon 146 (L146-12 bp). The deletion was confirmed by subcloning genomic PCR products and by sequencing. The deletion also was observed in four additional affected family members and was not observed in one unaffected family member (fig. 4).

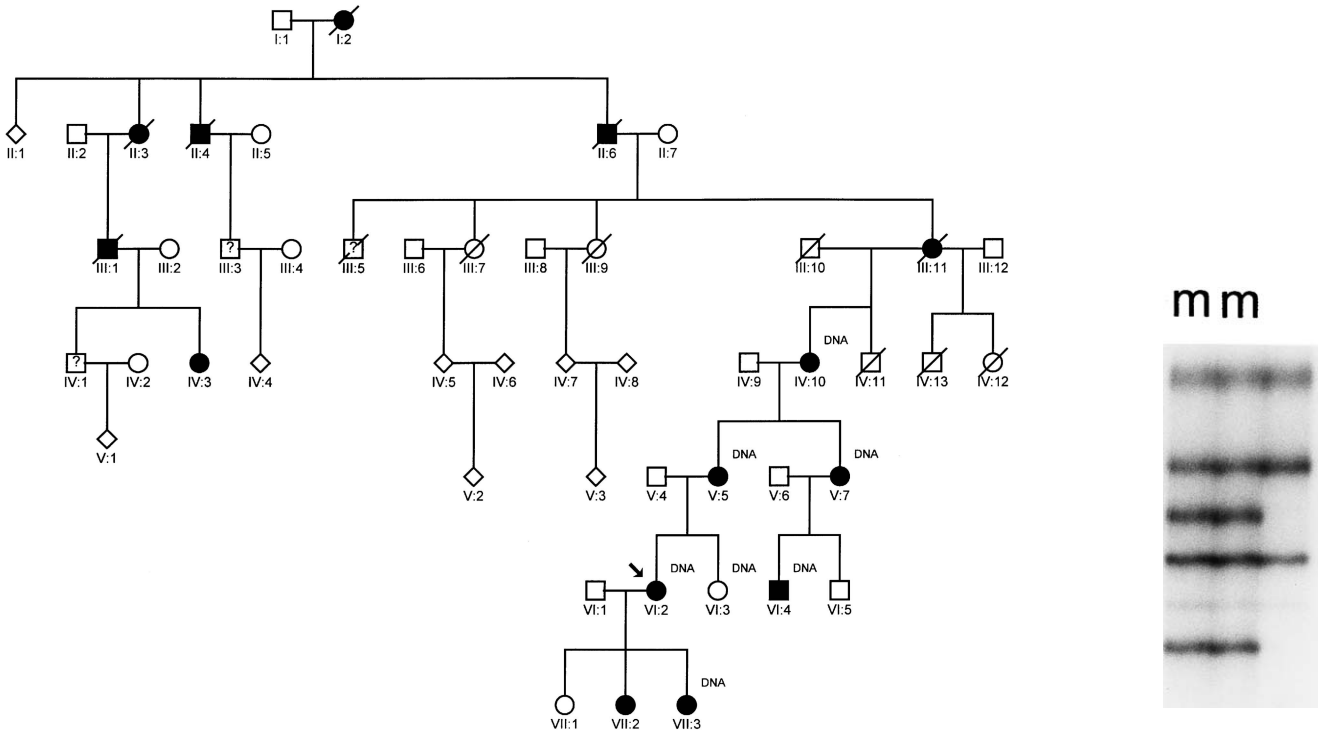

Figure 4 Family RFS900, Leber congenital amaurosis, autosomal dominant. *Left,* Pedigree. Blackened symbols represent affected individuals. A question mark (?) designates an individual of unknown phenotype. "DNA" to the upper right of the symbol indicates that a DNA sample was tested. *Right,* Results of SSCP analysis of exon 3a, showing aberrantly migrating fragments in the lanes designated "m."

Apparently Benign Variants

In addition to the four disease-causing mutations, two silent substitutions and two intronic variants were identified in this study. Three of the variants were observed in the affected proband of one family each. The first is a G→A transition in codon 183 (G183G), the second is a C→T transition in codon 199 (S199S), and the third is a G→A transition 15 bp upstream (5′) of the exon 3 coding sequence (IVS2-15G→A). No other family members were available for testing whether the substitution was cosegregating with the disease, in these families.

A fourth benign, intronic variant, an A→T transversion 65 bp upstream (5′) of exon 2 (IVS1-65A→T), was observed in affected members of six unrelated families. In one family (with autosomal dominant cone-rod dystrophy), this substitution was present in one affected individual but was not present in two other affected family members.

One plausible mechanism by which an apparently benign substitution may cause disease is through creation of a cryptic splice site. However, no alternate RNA splice site due to these substitutions was predicted by MacVector (Oxford Molecular Group). Also, silent substitutions are well tolerated in vertebrates, and the two intronic substitutions are far from the donor or acceptor splice sites. For these reasons, it is very likely that these are benign variants that do not contribute to the disease phenotype.

Clinical Description of Families with CRX *Mutations*

Family UTAD148, Cone-Rod Dystrophy.—There is complete penetrance in this large family with >32 affected members (Mintz-Hittner et al. 1975). The affected patients are symptomatic by age 8 years. Visual acuity drops rapidly, from 20/20 to 20/200, within a few months and then gradually decreases to no light perception by the patient's 50s or 60s.

A color-vision deficit occurs first in the blue-yellow spectrum (tritanomalous defect) but rapidly becomes complete. The visual fields initially demonstrate a relative central scotoma and constriction of the peripheral field. This progresses to dense centrocecal scotoma and marked constriction of the peripheral fields.

Fundus alterations begin with a decreased macular reflex, a metallic sheen to the macula, and normal peripheral retina. This is followed by progressive atrophy of the posterior neurosensory retina and of the retinal pigment epithelium, with marked bone spicule pigment in the peripheral retina, constriction of the retinal arterioles, and a waxy pallor of the optic disk.

In younger patients, the light-adapted electroretino-

Table 2

PCR Primer Sets for Amplification of SSCP Fragments of *CRX*

Amplified Fragment (Size [in bp])	Primer Sequences	Size(s) Analyzed (bp)
Exon 1 (257)	5′-CTGCACGTCACCCCATGGTGAGTAAC-3′ 5′-CAGAGGTCCTCCAAGAGATGAGGCC-3′	257
Exon 2 (314)	5′-GGATGGAATTCTTGGTCATCCCAC-3′ 5′-CTCTTTGTTCCGGGCAGGCCTC-3′	178, 136[a]
Exon 3a (501)	5′-CCAGCACCTCTCACCAATAAGTGTC-3′ 5′-GGCGTAGGTCATGGCATAGG-3′	191, 174, 136[a]
Exon 3b (294)	5′-CCTCCACAGATGTGTGTCCAGAC-3′ 5′-TGGGAGAAAGGTAGGGGTCTAGG-3′	294
Exon 3c (341)	5′-GCCTCCGCTTTCTGCTCTTC-3′ 5′-GCCCGATGGAGAGAGATGGAGACTG-3′	209, 132[a]

[a] Sizes after restriction digestion.

gram (ERG) is abnormal, but the scotopic ERG and the electro-oculogram (EOG) are normal. Gradually, the scotopic ERG and EOG become abnormal. Eventually, the ERG becomes unrecordable at all frequencies and intensities of stimulation in both light- and dark-adapted states, and the EOG is markedly reduced.

Family RFS014, Cone-Rod Dystrophy.—The primary visual disturbances in affected members of this family are poor color vision and uncorrectable visual acuity, which become evident by age 10 years and gradually worsen with time. At age 25 years, the proband had a best-corrected visual acuity of 20/50 OD and 20/200 OS (lower OS acuity was attributed to refractive amblyopia). Goldmann-Weekers dark-adapted thresholds (7° below fixation) were elevated by 0.3 log units. No field defects were evident on screening perimetry. Full-field ERGs revealed rod responses reduced by 50% and cone responses reduced by 90%. At age 27 years, acuity was further reduced to 20/160 OD and 20/320 OS, but no further decline was evident in the ERG.

The best-corrected visual acuity in the father of the proband, at age 54 years, was 20/200 OD and 20/250 OS. Goldmann-Weekers dark-adapted thresholds (7° below fixation) were elevated by 0.2 log units. Full-field ERGs revealed rod responses that were reduced by 85% and cone responses that were reduced by 99%. The deceased grandfather was thought to have had the same problem and was known to have had low visual acuity and color blindness prior to his death at age 59 years.

Family RFS087, Retinitis Pigmentosa (*Late Onset with Cone-Rod Involvement*).—Affected patients in this family were asymptomatic until their 50s or 60s. At age 58 years, the proband was found to have a temporal-field defect in each eye, associated with pigment clumping in the nasal periphery. Visual acuity was 20/15 OD and 20/20 OS. The dark-adapted threshold (7° below fixation) was elevated 0.2 log units. Rod ERG amplitudes were 60% lower than normal, while cone ERGs to 30-Hz flicker were reduced by 65%. The proband has shown significant progression over 14 years of follow-up. On the most recent visit, at age 72 years, visual acuity was 20/600 OD and 20/900 OS, and the dark-adapted threshold was elevated 0.8 log units. Both rod and cone ERGs showed significant progression and were reduced by 90%.

The proband's father died at an early age, and nothing is known about his visual status. The proband's son was asymptomatic at age 44 years, with visual acuity of 20/20 in both eyes. Dark-adapted thresholds were normal. Fluorescein angiography revealed slight peripheral granularity of the epithelium, without narrowing of vessels or optic-nerve pallor. Rod ERG amplitudes were 50% lower than normal, while cone ERGs to 30-Hz flicker were reduced by 55%. Five years later, at age 49 years, he had become aware of distortions and occasional blind spots. Visual acuity was 20/20 OD and 20/25 OS, the dark-adapted threshold was still normal, and full-field ERGs did not show significant progression.

Family RFS900, Leber Congenital Amaurosis (*Early-Onset Retinitis Pigmentosa*).—Affected members of this family have severe visual disturbances in infancy, accompanied by nystagmus; some family members are severely affected from birth, whereas other affected members have partial vision into midlife (Heckenlively 1988). The proband (IV:5 in fig. 4) was seen at age 27 years, with best-corrected acuity of 20/300 OD and 20/80 OS. His rod ERG was undetectable, and his cone ERG was barely detectable (0.1 μV). His Humphrey field with spot size 3 had an equivalent diameter of 6°. His fundus showed attenuated vessels, clumped pigment, and retinal pigment epithelium atrophy, including the macula. At age 11 years, his acuity was 20/200 OD and 20/60 OS.

His mother (III:3 in fig. 4) was seen at age 46 years. She retains only light perception in each eye. Her rod and cone ERG responses are undetectable. She was unable to see the most intense Goldmann-Weekers test target (elevated >6 log units) and was unable to perform a visual field. She showed severe vessel attenuation, RPE

Table 3

***CRX* Mutations and Sequence Variants in this Population**

Codon[a]	Mutation	cDNA[b]	Phenotype	Family or Frequency (%)
41	Arg41Gln (R41Q)	G121A (CGG→CAG)	Retinitis pigmentosa, autosomal dominant	RFS087
80	Glu80Ala (E80A)	A238C (GAG→GCG)	Cone-rod dystrophy, autosomal dominant	UTAD148
146	Leu146-12 (L146-12)	436del12	Leber congenital amaurosis, autosomal dominant	RFS900
196	Ala196+1 (A196+1)	585insC (GCC→GCCC)	Cone-rod dystrophy, autosomal dominant	RFS014
(83)	IVS2-15G→A	253-15G→A	Benign variant	<.1
(34)	IVS1-65A→T	101-65A→T	Benign variant	.2
183	Gly183Gly (G183G)	G538A (GGG →GGA)	Silent substitution	<.1
199	Ser199Ser (S199S)	C596T (TCC→TCT)	Silent substitution	<.1

[a] Parentheses around a codon indicate the nearest codon to an intronic sequence variant.
[b] cDNA sequence numbering based on GenBank accession number AF024711 (Freund et al. 1997), counting the first nucleotide of the first codon as 1.

granularity, and patchy RPE atrophy (including a macular lesion) and has posterior cataracts. As determined by her history, she had low vision from infancy. At age 30 years, her visual acuity was 2/200 OD and 20/200 OS, and her Goldmann visual field with the I-V isopter was 8° OD and 12° OS.

The 30-year-old cousin of the proband (IV:1 in fig. 4) also had only light perception in each eye. She had extremely low acuity and night blindness from early infancy. Her rod and cone ERG responses were undetectable. She was unable to see the DA target (elevated >6 log units) and was unable to perform a visual field. Fundus examination revealed severe vessel attenuation, granularity of the RPE, and atrophy of both maculae.

Her daughter (V:3 in fig. 4) was seen at age 20 mo. Her binocular visual acuity, measured with forced-choice preferential looking, was 20/300. She had grossly abnormal rod sensitivity, determined by steady-state pupilometry. She had nystagmus soon after birth, similar to other affected family members. The posterior pole was unremarkable, with the possible exception of slight vessel attenuation.

Individual III:2 (fig. 4) was seen at age 49 years, with no light perception in either eye. At age 35 years, she was reported to have no light perception OD and barely any light perception OS. Her fundus showed severe vessel attenuation and diffuse RPE pigmentary disturbances, including macular atrophy.

The grandmother (II:2 in fig. 4) of the proband was age 67 years at examination. She had no light perception in either eye. She reported having visual difficulty, including nystagmus, from infancy, although she could read until age 15 years. On examination at age 52 years, she was found to have barely any light perception in each eye, posterior subcapsular cataracts, moderate vessel attenuation, and diffuse RPE atrophy.

Discussion

Previous studies associated mutations in the *CRX* gene sequence with cone-rod dystrophy and de novo Leber congenital amaurosis (Freund et al. 1997; Swain et al. 1997; Freund et al. 1998). We now extend the range of phenotypes that may result from mutations in CRX to include dominant retinitis pigmentosa and dominant Leber congenital amaurosis. A diagram of the known disease-associated mutations in *CRX* is shown in figure 5. Thus, like those in peripherin/*RDS,* mutations in *CRX* are associated with several forms of retinal degeneration.

In a survey of 294 unrelated affected individuals from families with various forms of inherited retinopathy, we found four disease-associated mutations in *CRX*. These mutations were not observed in any of the other unrelated individuals screened, a total of 588 chromosomes, nor in other published *CRX* surveys, except in association with inherited retinopathy. Thus, CRX mutations are a rare but significant cause of inherited retinal diseases.

Are these four *CRX* mutations the cause of disease in the families in which they are found? The Glu80Ala (E80A) mutation found in the family with autosomal dominant cone-rod dystrophy, UTAD148, is segregating with the disease phenotype in 35 informative family members; the disease locus is also tightly linked to the *CORD2* locus on 19q13 (Sohocki et al. 1997). The E80A mutation was previously reported in another large family with cone-rod dystrophy (Freund et. al 1997), although haplotype analysis shows that the mutations are of independent origin. This mutation falls in the recognition helix of the homeodomain in the CRX protein and may be involved in dimerization (Freund et al. 1997). The change of a glutamic acid residue to an alanine is likely to disrupt the salt bridge between residues

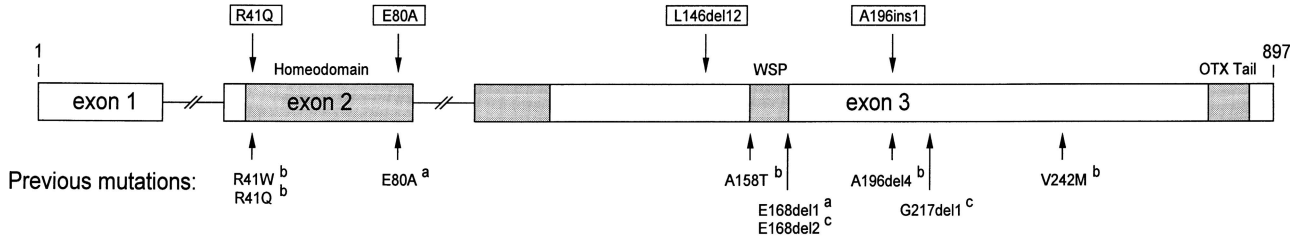

Figure 5 Disease-associated mutations within *CRX*. Nucleotide numbering of the cDNA begins with the A of the ATG start codon, assigned as position 1. Mutations reported in this article are listed above the cDNA diagram, and previously reported mutations are listed below the cDNA diagram. a = reported in Freund et al. 1997, b = reported in Swain et al. 1997, and c = reported in Freund et al. (1998).

R69 and E80, probably causing a loss of function (Freund et al. 1997). For these reasons, it is highly likely that this is the cause of disease in both families.

The 1-bp insertion (A196+1) found in RFS014, the second family in this series with dominant cone-rod dystrophy, is present in four affected individuals. The insertion causes a frameshift leading to 39 altered amino acids, followed by a premature stop codon. The result of the stop codon is a protein truncated by 22%, lacking the conserved OTX tail. This mutation is likely to cause loss of function and to lead to the disease seen in this family.

The Arg41Gln (R41Q) mutation is found in three affected family members and is absent from one unaffected at-risk member of RFS087, a family with autosomal dominant retinitis pigmentosa. The same mutation was reported previously in a family with cone-rod dystrophy (Swain et al. 1997); clinical details have not been published. The clinical phenotype in RFS087 is consistent with late-onset retinitis pigmentosa or mild, atypical, cone-rod dystrophy, but a direct clinical comparison of the two families is necessary in order to determine whether they actually differ in phenotype. Substitution of a tryptophan for the arginine at codon 41 produces a recombinant CRX homeodomain with reduced binding activity for the rhodopsin promoter (Swain et al. 1997). Since the substitution of glutamine for arginine at this site causes a nonconservative change in net charge, this mutant CRX protein may also affect promotor activity. For these reasons, this mutation is likely to be the cause of the clinical phenotype in both families.

Finally, the 12-bp deletion (L146-12) found in the family with dominant Leber congenital amaurosis, RFS900, has not been reported previously. The mutation was observed in six affected family members. The mutation results in an in-frame deletion of residues 147–150. (The codon for residue 146, a leucine, is also affected, but the resulting triplet still codes for leucine.) These four amino acids are also present in the mouse Crx protein. Such a substantial deletion is likely to affect the function of the CRX protein. For these reasons, this mutation is likely to be the cause of the clinical phenotype in this family.

The four *CRX* mutations found in this survey account for a wide range of clinical phenotypes. The E80A mutation is found in families with classic, dominant cone-rod dystrophy, including symptoms by age 8 years, color-vision deficits, and early cone involvement followed by rod involvement in later years. The A196+1 mutation is associated with a progressive loss of visual acuity that begins in the 1st decade of life. This mutation produces a cone-rod pattern of progressive retinal degeneration, but patients retain both rod and cone ERG responses well into the 5th decade. The R41Q mutation was found in one family, RFS087, with an initial diagnosis of autosomal dominant retinitis pigmentosa. Although patients in this family were thought to have retinitis pigmentosa on the basis of fundus appearance, affected family members are asymptomatic until their 50s or 60s, retain detectable rod ERG responses at least into their 70s, and have comparable loss of rod and cone ERG responses. The L146-12 mutation is associated with a more severe retinal dystrophy that shares many of the characteristics of Leber congenital amaurosis, including an undetectable ERG in the 1st decade of life.

Mutations in *CRX* are associated with clinical characteristics of cone-rod dystrophy, dominant retinitis pigmentosa, or dominant Leber congenital amaurosis. Different *CRX* mutations may produce significantly different retinal disease phenotypes, and these diseases also have significantly different ages at onset. Thus, mutations in CRX, a transcriptional activator of several retinal genes, may be associated with congenital, early-onset, or late-onset retinal degeneration.

Acknowledgments

We thank Dr. Shomi Bhattacharya, Department of Molecular Genetics, Institute of Ophthalmology, London, for providing DNA samples from the Greek family with cone-rod

dystrophy. This work was supported by the Foundation Fighting Blindness and the George Gund Foundation, Research to Prevent Blindness, the Farish Fund and the M. D. Anderson Foundation, National Institutes of Health grants EY07412 and EY05235, Institutional National Research Service Award EY07024 from the National Eye Institute–National Institutes of Health, and grants from the Medical Research Council of Canada and the Retinitis Pigmentosa Foundation of Canada. R.R.M. is an International Research Scholar of the Howard Hughes Medical Institute.

Electronic-Database Information

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

GenBank, http://www.ncbi.nlm.nih/Web/Genbank/ (for *CRX* cDNA sequence [AF024711])
Lawrence Livermore National Laboratory, http://www.llnl.gov/ (for human chromosome 19 sequences)
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.gov/Omim (for CRX [MIM 120970]
Retinal Information Network (RetNet), http://www.sph.uth.tmc.edu/RetNet (for cloned and/or mapped genes causing inherited retinal degeneration in humans)

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