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Genetics

Mutation spectrum of the gene encoding the β subunit of rod phosphodiesterase among patients with autosomal recessive retinitis pigmentosa

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ABSTRACT **Mutations in the gene encoding the β subunit of rod cGMP phosphodiesterase are known causes of photoreceptor degeneration in two animal models of retinitis pigmentosa, the *rd* (retinal degeneration) mouse and the Irish setter dog with rod/cone dysplasia. Here we report a screen of 92 unrelated patients with autosomal recessive retinitis pigmentosa for defects in the human homologue of this gene. We identified seven different mutations that cosegregate with the disease. They were found among four patients with each patient heterozygously carrying two mutations. All of these mutations are predicted to affect the putative catalytic domain, probably leading to a decrease in phosphodiesterase activity and an increase in cGMP levels within rod photoreceptors. Mutations in the gene encoding the β subunit of rod phosphodiesterase are the most common identified cause of autosomal recessive retinitis pigmentosa, accounting for ≈4% of cases in North America.**

Retinitis pigmentosa (RP) is a group of human hereditary retinal degenerations that affects between 50,000 and 100,000 people in the United States (1, 2). Early stages of the disease are characterized by night blindness and constricted visual fields. In later stages affected patients typically show abnormal accumulation of pigment in the midperipheral retina where rod photoreceptors are normally at highest density. Light-evoked electrical responses from the retina, recorded as electroretinograms (ERGs), are abnormal (3, 4). RP can be inherited as an autosomal dominant, autosomal recessive, or X chromosome-linked disease. Furthermore, within each of these genetic categories there is nonallelic heterogeneity. The course of the disease has been slowed with oral vitamin A supplementation (5).

Previous reports have implicated defects in either of two members of the phototransduction cascade, rhodopsin or the β subunit of rod cGMP phosphodiesterase (PDE-β), as the cause of some cases of RP (6, 7). Initiation of phototransduction begins when rhodopsin absorbs a photon of light. Photoexcited rhodopsin then complexes with a rod G protein called transducin. The activated α subunit of transducin, in turn, activates rod phosphodiesterase (PDE) by releasing the inhibitory PDE γ subunit from the catalytic complex composed of a PDE α subunit and a PDE-β. Activated PDE hydrolyzes cGMP; low cGMP levels lead to closure of cation channels in the cell membrane and hyperpolarization (8). There is evidence suggesting that either disruption of the phototransduction pathway or continuous activation of it may lead to photoreceptor cell death in humans (9, 10).

Mutations in the PDE-β gene can cause photoreceptor degeneration in both mice and dogs (11–14). Here we describe a comprehensive screen of 92 unrelated patients with autosomal recessive RP for mutations in the PDE-β gene. Tabulating the spectrum of mutations in the PDE-β gene that causes autosomal recessive RP is important for understanding the relationship between rod PDE and RP. Discovery of additional mutations can give insight into the structural and functional domains of PDE-β and may thereby provide the basis for future *in vitro* studies. Identifying a cohort of patients who share defects in the same gene will allow for clinical studies of them and their unaffected carrier relatives that may identify clinical features helpful in diagnosis. Because these patients have defects in the same gene as the *rd* (retinal degeneration) mouse and the Irish setter dog with rod/cone dysplasia, they may benefit most from research on these animal models.

METHODS

Ascertainment of Patients. We recruited 142 unrelated patients with autosomal recessive RP. For various reasons including poor amplification of DNA and limited quantities of DNA, only 92 patients were completely screened. In addition, we recruited 82 unaffected individuals without a family history of RP to serve as controls. Informed consent was obtained from all participants. Each affected patient had unaffected parents and at least one affected sibling or were the offspring of a consanguineous mating. If the patient and the affected sibling were both male, an ophthalmologic exam and an ERG of the mother were done to rule out the possibility of X chromosome-linked disease (15). All affected patients had reduced and delayed or absent rod ERG responses. Four of the patients had "elevated cGMP-type" retinal degeneration characterized by ERG responses compatible with presumed PDE inhibition (16). No patient included in our screen had defects in the rhodopsin gene as detected by single-strand conformation polymorphism (SSCP) analysis (9). Leukocyte nuclei were prepared from between 10 and 50 ml of venous blood as described (17). These nuclei were stored at −70°C before DNA purification and analysis.

Screening for PDE-β Mutations. We used Southern blot analysis to screen for rearrangements and deletions of the PDE-β gene. Southern blots were probed with human PDE-β cDNA clones (provided by M. Hayden, University of British Columbia, Vancouver) after labeling with ^{32}P. One probe, AR-1′, extended from position 1 to 1527, and a second probe, AR-2RM1.2, extended from position 1528 to 2717 (18). Genomic DNA from each patient was digested with the restriction endonuclease *Tth*111I for probing with AR-1′ and separately digested with *Eco*RI for probing with AR-2RM1.2. Digested DNA fragments were separated by electrophoresis

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Abbreviations: ERG, electroretinogram; PDE, phosphodiesterase; PDE-β, β subunit of rod cGMP PDE; RP, retinitis pigmentosa; SSCP, single-strand conformation polymorphism; RFLP, restriction fragment length polymorphism.

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through 0.8% agarose gels and transferred to nylon membranes before hybridization with the probes.

We used SSCP analysis to screen for variant bands with oligonucleotide primers based on the sequence of Weber *et al.* (19) and Riess *et al.* (20) (see Table 1 for the sequences of the oligomers and the conditions for PCR amplification of each primer pair). Five of the amplified DNA fragments were digested with restriction endonucleases to yield smaller fragments for SSCP analysis (Table 1). PCR amplified DNA was heat-denatured, and single-stranded fragments were separated by electrophoresis at room temperature through two 9% polyacrylamide gels, one with and one without 10% (vol/vol) glycerol. Gels were run at 6, 12, or 30 W for 6 to 16 hr at room temperature before drying and autoradiography. Except for variant bands in exon 22, variant bands were analyzed by sequencing PCR-amplified DNA as described (21) (oligomers and conditions used for DNA sequencing are available upon request). DNA from patients with variant bands in exon 22 was amplified by PCR using the following primers: sense 5′-ACCAGGCAGGAGGGAAGGAA-3′; antisense 5′-GGCTTCCTAACCTCTTGTAG-3′ (yielding 232-bp DNA fragments). The DNA fragments were cloned into the plasmid, pBluescript II TKS (22), after the plasmid had been cut with *Eam*1105I. The sequencing reactions were done by using the Sequenase kit (United States Biochemical) and standard methods.

RESULTS

We examined the PDE-β gene in 142 unrelated patients with autosomal recessive RP, of whom 92 were comprehensively screened exon-by-exon. Southern blot analysis of the PDE-β locus failed to identify any rearrangements or deletions (data not shown). Although variant *Tth*111I fragments were detected with the cDNA probe AR-1′, two pieces of data suggest that they represented *Tth*111I restriction fragment length polymorphisms (RFLPs). (*i*) The frequency of each variant fragment was the same in the group of patients with autosomal recessive RP and in a group of 82 normal individuals. (*ii*) No corresponding variant bands were seen when the genomic DNA was cut with *Bam*HI or *Eco*RI and probed with AR-1′. No variant *Eco*RI fragments were detected with cDNA probe AR-2RM1.2.

Using SSCP analysis, we found a total of 23 variant bands due to 25 sequence variations. DNA sequencing revealed four silent changes in the coding sequence (Ser-291 → Ser, Thr-305 → Thr, Arg-705 → Arg, Gly-842 → Gly), eight intron sequence variations, and two sequence variations in the 3′ untranslated region. Four other sequence variations led to the amino acid changes (Ala-155 → Ser, Glu-166 → Lys, Tyr-219 → His, and Arg-602 → His). Tyr-219 → His and Arg-602 → His did not cosegregate with RP in the respective families. Cosegregation analysis could not be done for Ala-155 → Ser and Glu-166 → Lys because essential family members were unavailable. Both amino acid changes are located in the amino terminus in a region outside the putative catalytic domain within residues 555–792 (19, 23, 24). Table 2 lists the sequence variations that appear unrelated to RP and their frequencies.

The remaining 7 of 25 sequence variations affected the coding sequence and cosegregated with autosomal recessive RP. Four of these mutations (Gln-298 → X, Pro-496(1-bp del), Arg-531 → X, His-557 → Tyr) have been reported by us (7). The three additional mutations were an A → T transversion at position 21887 in exon 17, resulting in a nonsense mutation (Lys-706 → X) in patient AR120 (Fig. 1*A*); a T → C transition at position 18075 in exon 12, resulting in a missense mutation (Leu-527 → Pro) in patient AR90 (Fig. 1*B*); and a G → A transition at position 22624, altering the splice donor site of intron 18 also in patient AR90 (Fig. 1*C*) [position numbers are according to the genomic sequence of Weber *et al.* (19)].

Lys-706 → X was the second mutation to be found in patient AR120. We previously reported that AR120 heterozygously carried Gln-298 → X and that this mutation cosegregated with RP. SSCP analysis of DNA from the relatives of AR120 (family no. 6235, Fig. 2*A*) revealed that the two affected family members carry both Gln-298 → X and Lys-706 → X. Their father is heterozygous for Gln-298 → X, whereas their mother is heterozygous for Lys-706 → X, indicating that the mutations

Table 1. Oligomers and conditions used for PCR amplification of exons of the human PDE-β gene

Exon	Primer pairs (sense/antisense, 5′ to 3′ direction)	Amplified fragment size, bp	pH	[$MgCl_2$], mM	Annealing temperature, °C	Restriction endonuclease
5′-UTR	CGCTAGAAGCCAAATGCCTG/GCGGGCAAAATCGGGGTTCT	239	8.6	0.75	63	*Hha* I
1a	GGACCAGAACCCCGATTTTG/GTTGCGCTGGCGGTACATGA	268	8.4	1.00	60	*Ban* I
1b	GCGTGGTCTTCAAGGTCCTG/TCCGAGCTGACCCTTAGACA	326	8.4	1.00	60	*Hin*fI
2	CGGTGCGACAGCCTCTTTAG/GGGCAGGCAGGCAGGGAGGC	250	8.4	1.00	57	
3	CACGGGGGCACATGTCTGAA/GGACCCTGGCCTCGTGGGCA	195	8.6	1.00	63	
4	CCCTGCTGCTGTGGTCAGAC/AGAGAAGAGGTGAGGGTGGG	292	8.4	1.00	60	*Taq*$^{\alpha}$I
5	ACCGCCCCACCCTCACCTCT/CCCTATCCCTCCCTCTCCTG	181	8.4	0.75	63	
6	CAGCCCCCCGACCAGTGTCTTCTGC/GGGCGCTGAGCCGGGGTCTGAGGGG	166	8.6	1.25	63	
7	GCCTATCTGACCCCTGCTCTCTGCC/GAGAAGCACACATCGCCGCACCCAG	164	8.6	0.50	66	
8	GGCCACAGAGGCCATTTTAGATCAT/CATCGCTGAATCAAAATCTCCTCTT	190	8.6	2.00	63	
9	CCAGGGCCAGCCTCAGGGCG/CCGGCGGAACCACGTCTGCC	230	8.4	1.00	60	
10	GGGAGAGGACGACAAAGGGC/CTGACGTCCTGCCCCGCCGC	356	8.4	1.25	63	*Dde* I
11	GAAGCTCTTTCTCGTGACAC/AACTCTTCCTCCCAGCCTCA	169	8.6	1.00	48	
12	CCTCCCTCAGCCCACAATCC/TGACTATGCGCCTGCGGTGT	203	8.4	1.00	63	
13	GCGCTCTGGCGGGACTTACA/GCCTGCACAACCCTGGTGAT	204	8.4	1.00	63	
14	CTGGAGCCAGGACCGGTGAG/GGCTAATTCACATGCCCGCC	186	8.6	0.75	63	
15	CCCACGGGCCTCACCTCCAC/AGCAGAAACCGAGGGTGAGT	187	8.4	1.25	60	
16	CCTGACGCCCTGGGCATAAC/GGCGCCCCCTCCCGGAAGGC	157	8.6	1.00	60	
17	GGGGCGGGGTCTCCACACTT/GGACCCCTCGTGCCCCCTCC	196	8.4	0.75	60	
18	CAGCACTCGTGCCCGGTTTG/TGGCCTGGCCCCGAGGTGGC	225	8.4	1.00	60	
19	GCTCAACCGGAGCCTGTGTG/AGAACCCCCTGCCTGAGACC	315	8.6	1.25	60	*Pvu* II
20	CTGTTCTCCCGCCCTCTGTT/TTGCCTGCTCCCTTGCCTGC	194	8.6	1.00	50	
21	GACTGGTGGTGACTTCTCGA/AGAGTCACCGAGTCCCTGCC	224	8.6	1.25	60	
22	ACCAGGCAGGAGGGAAGGAA/GGTGAAGATTGAGGGAGCCA	177	8.4	0.75	48	

5′-UTR, 5′ untranslated region; exon 1 was amplified in two segments named 1a and 1b.

Table 2. DNA polymorphisms and rare variants in the PDE-β gene

Exon	Sequence	Amino acids	Chromosomes with sequence,* no.
1‡	GCC → TCC	Ala-155 → Ser	1
2†‡	GAG → AAG	Glu-166 → Lys	7
3	TAC → CAC	Tyr-219 → His	2
5‡	TCT → TCC	Ser-291 → Ser	1
5†‡	ACG → ACA	Thr-305 → Thr	12
6†‡ (intron 6)	CCCCGGGGG → CCCCCGGGG		19
10‡§ (intron 9)	CTGCGACAC → CTGTAACAC		3
10‡ (intron 9)	GGAGGGGGC → GGAGAGGGC		1
11†‡ (intron 11)	CGGTCCCTC → CGGTTCCTC		10
11‡ (intron 11)	GCCACGTGT → GCCATGTGT		1
14	CGC → CAC	Arg-602 → His	1
17¶	CGG → CGA	Arg-705 → Arg	1
19‡ (intron 19)	TTCCACTCC → TTCCTCTCC		6
20 (intron 20)	GTTCCGAGC → GTTCTGAGC		1
22†‡	GGC → GGT	Gly-842 → Gly	2
22†‡ (3′ UTR)	TCCCATGGG → TCCCGTGGG		ND
22‡ (3′ UTR)	GGACCCTAT → GGACACTAT		ND

¶ND, not determined. UTR, untranslated region.
*The 184 chromosomes were surveyed from a set of 88 patients with autosomal recessive RP and 4 patients with "elevated cGMP-type" retinal degeneration who underwent screening of all exons.
†DNA polymorphisms and rare variants that have been published (20, 25, 26).
‡No cosegregation analysis was done.
§The published sequence of intron 9 (CTGCACGATG) (19) is incorrect and should read CTGCGACACCATG.
¶This silent change is syntenic with the nonsense mutation, Lys-706 → X, in family no. 6235 (Fig. 1*A*).

are allelic. If expressed, the Lys-706 → X mutation is predicted to produce a truncated PDE-β protein missing a portion of the putative catalytic domain as well as the C-terminal domain required for posttranslational processing and membrane association (19, 23, 24, 27, 28).

In family no. 4151, patient AR90 and his affected brother heterozygously carry both Leu-527 → Pro and the splice donor-site mutation in intron 18 (Fig. 2*B*). The father heterozygously carries Leu-527 → Pro, while the mother heterozygously carries the splice donor-site mutation, indicating that these two mutations are also allelic. The presence of Pro-527, an amino acid residue that can cause peptide chain bending, is likely to disrupt the putative catalytic domain. There are many ways in which the splice donor-site mutation might affect PDE-β, such as skipping an exon or including an intron in mRNA.

Like the patients we described previously with PDE-β mutations, AR90 and his affected brother had clinical findings typical of RP. They reported absent night vision since early childhood. Their best-corrected visual acuity was 20/40 or better, and their visual fields were constricted to ≈20° diameter with or without peripheral islands. Final dark-adapted thresholds were elevated 2 or more logarithmic units, consistent with absent rod function. Ophthalmoscopy revealed attenuated retinal vessels and typical intraretinal bone-spicule pigment around the midperiphery. ERG recordings did not reveal any rod-isolated responses to dim blue light. Responses to single flashes of white light were markedly reduced in amplitude, indicating loss of both rod and cone function. Cone-isolated responses to white flickering light, monitored with computer averaging, were reduced and delayed.

No mutations in the PDE-β gene were found in four unrelated patients with "elevated cGMP-type" retinal degeneration included among the 92 patients who were comprehensively screened exon-by-exon. These patients have ERGs that are similar to those observed in the isolated cat eye perfused

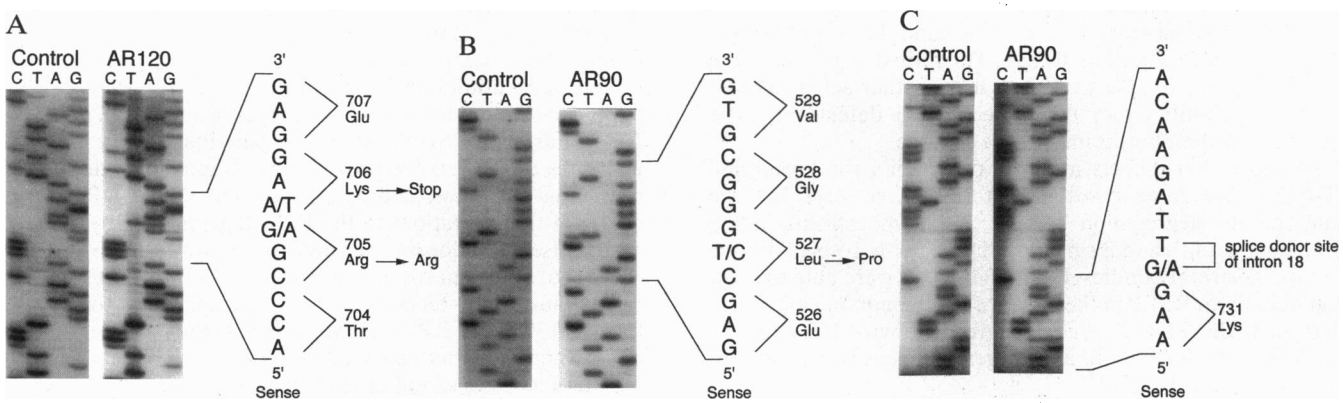

FIG. 1. Nucleotide sequence of novel PDE-β mutations in patients with autosomal recessive RP. (*A*) Patient AR120 is a heterozygous carrier of a G → A transition in the third base of codon 705 that does not cause an amino acid change and an A → T transversion in the first base of codon 706 that results in a nonsense mutation. DNA sequencing of the parents of AR120 revealed that the mother, I-2 in family no. 6235, heterozygously carries both sequence variations, indicating that they are syntenic (data not shown). (*B* and *C*) Patient AR90 is a heterozygous carrier of (*i*) a T → C transition in the second base of codon 527 that results in a missense mutation and (*ii*) a G → A transition that alters the splice donor site of intron 18.

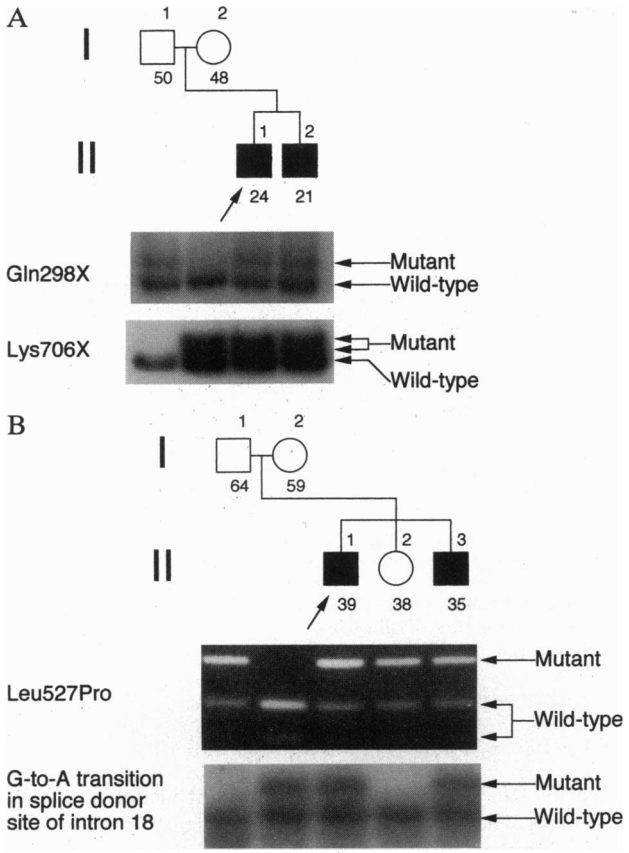

FIG. 2. Cosegregation of autosomal recessive RP with variant bands detected by SSCP analysis or restriction endonuclease digestion. (*A*) SSCP analysis of DNA from family no. 6235. Patient AR120 with the mutations Gln-298 → X (7) and Lys-706 → X (Fig. 1*A*) is member II-1 (arrow). (*B*) Cosegregation of Leu-527 → Pro with RP in family no. 4151 is shown by digesting the PCR-amplified DNA fragments of exon 12 with *Alu* I. Family members without the Leu-527 → Pro mutation have DNA fragments of 102 bp, 62 bp, and 39 bp (the 39-bp fragment is not seen here). Family members who heterozygously carry the Leu-527 → Pro mutation have the wild-type DNA fragments as well as a DNA fragment of 164 bp, resulting from destruction of the *Alu* I site at position 18075 by the mutation. Cosegregation of the splice donor-site mutation with RP is shown by SSCP analysis. Patient AR90 is member II-1 (arrow) of family no. 4151. The number below each symbol is the age of the individual in February, 1994. Results of SSCP analysis and restriction endonuclease digestion are aligned with pedigree symbols. Arrows at right point to mutant and wild-type bands.

with the PDE inhibitor, isobutylmethylxanthine (16), and therefore these patients were *a priori* candidates for carrying null mutations in the PDE-β gene. The retinal degeneration in these cases may be due to defects in the other active subunit of PDE (subunit α) or could be due to defects in other members of the phototransduction cascade.

To prove that defects in genes other than rhodopsin and PDE-β cause some cases of autosomal recessive RP, we analyzed the segregation pattern of polymorphisms in the genes encoding rhodopsin and PDE-β with respect to the disease in several families. In one family we were able to show that neither an RFLP in the rhodopsin gene nor an amino acid change in the PDE-β gene cosegregated with RP (Fig. 3), indicating that a defect in a third genetic locus is the cause of the disease in this family.

DISCUSSION

After a comprehensive screen using both Southern blotting and SSCP analysis of the entire coding region, we identified a total of seven mutations in the PDE-β gene that cosegregate

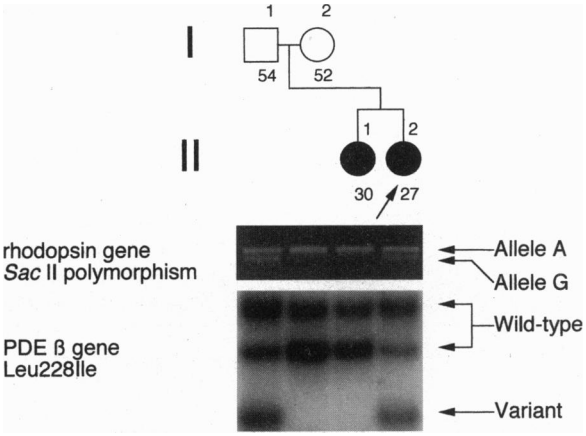

FIG. 3. Exclusion of defects in the rhodopsin gene and in the PDE-β gene as the cause of autosomal recessive RP in family no. 6829. Restriction endonuclease digestion of exon 1 of the rhodopsin gene and SSCP analysis of exon 3 of the PDE-β gene show that neither the rhodopsin *Sac* II RFLP nor the PDE-β amino acid change, Leu-228 → Ile, cosegregate with the disease. The rhodopsin *Sac* II RFLP is located in the 5′ untranslated region (29). The PDE-β amino acid change Leu-228 → Ile was identified in patient AR95 (arrow). Leu-228 → Ile is not listed in Table 2 because the coding sequence of the PDE-β gene has only been partially screened in this patient. The number below each symbol is the age of the individual in February, 1994. Results of SSCP analysis and restriction endonuclease digestion are aligned with pedigree symbols.

with autosomal recessive RP. Three of these are nonsense mutations (Gln-298 → X, Arg-531 → X, and Lys-706 → X), two are missense mutations (Leu-527 → Pro and Tyr-557 → His), one is a frameshift mutation [Pro-496(1-bp del)], and one alters the splice donor site in intron 18. All of the mutations are predicted to disrupt the enzymatic function of PDE-β, suggesting that a deficiency in PDE activity is the primary defect leading to photoreceptor cell death in the respective patients. A decrease in PDE activity in the outer retina and a corresponding increase in cGMP levels has been seen in two animal models with retinal degeneration due to nonsense mutations in the PDE-β gene: the *rd* (retinal degeneration) mouse, homozygous for Tyr-347 → X (12, 30, 31) and the Irish setter dog with rod/cone dysplasia, homozygous for Trp-807 → X (13, 14). High concentrations of cGMP have been proposed to be deleterious to photoreceptors (32, 33); however, the precise mechanism that leads to photoreceptor cell degeneration due to null mutations of PDE-β remains unclear.

The seven mutations in the human PDE-β gene were found in four unrelated patients with each patient heterozygously carrying two mutations. Because these four patients were found after a comprehensive screen of 92 cases and a partial screen of 50 additional cases, mutations in the PDE-β gene appear to account for ≈3–4.5% of cases of autosomal recessive RP in our set of North American patients. On the basis of estimates of the relative proportions of autosomal dominant, autosomal recessive, and X chromosome-linked RP (2), we calculate that mutations in the PDE-β gene cause ≈1–2% of all RP cases. Rhodopsin gene defects, known to cause 20–30% of cases of autosomal dominant RP (29, 34–36) and at least one case of autosomal recessive RP (9), are calculated to account for ≈9–13% of all RP cases. Mutations in the peripherin/*RDS* gene, which cause autosomal dominant RP, dominant retinitis punctata albescens, and other forms of photoreceptor degeneration with early involvement of the macula (37–42), are estimated to cause ≈0.5–2% of all RP cases. Thus, mutations in the PDE-β gene are the most common identified cause of autosomal recessive RP and account for a similar proportion of RP cases (summing all genetic types), as do mutations in the peripherin/*RDS* gene.

Although unlikely, it was possible that all cases of autosomal recessive RP were due to mutations in the rhodopsin and PDE-β genes. Mutations may not have been detected if they were in regions of the transcriptional unit that were not screened—e.g., the promoter or the introns. By performing cosegregation analysis with polymorphisms in the rhodopsin gene and in the PDE-β gene, we have shown that a defect in a third genetic locus is the cause of autosomal recessive RP in at least one family, proving that at least one other locus can cause recessive RP. Thus, like dominant and X chromosome-linked RP, recessive RP is also heterogeneous. One explanation for the nonallelic heterogeneity is that recessive RP is due to defects in different members of a biochemical pathway unique to photoreceptors. Because defects in the rhodopsin gene and in the PDE-β gene have already been found, the phototransduction cascade may be a biochemical pathway targeted by other autosomal recessive RP genes. The human genes encoding several other members of this pathway have been isolated and are available for candidate gene analysis. In view of the small proportion of patients observed so far having disease due to any particular gene, searches for mutations among these candidate genes will probably require a large number of well-characterized patients to evaluate their potential role in RP.

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