noradrenergic receptor activation.

Our data show that catecholamines from sweat gland innervation induce production of a cholinergic differentiation factor in developing sweat glands, thereby triggering the switch to a cholinergic phenotype. Further, they indicate that the initial expression of catecholamines by cholinergic sympathetic neurons is not simply a default pathway (4) but is an essential step in their developmental differentiation. The requirement for target innervation, which is not necessary for production of nerve growth factor (19), the target-derived trophic factor for sympathetic neurons, may restrict the availability of the differentiation factor and increase the specificity of neuron-target interactions.

This study shows that both anterograde and retrograde signaling are needed for development of functional synapses between sympathetic neurons and sweat glands. Noradrenergic neurons contact the glands and stimulate production of SGF. Sweat glands release SGF, which induces cholinergic properties in the noradrenergic neurons, and acetylcholine then induces maturation of the sweat glands (20), which results in the establishment of a functional synapse.

## **REFERENCES AND NOTES**

- .. S. Landis, Dev. Biol. 77, 348 (1980); R. Mains and
- P. Patterson, J. Cell Biol. 59, 329 (1973). 2. P. Patterson and L. Chun, Dev. Biol. 56, 263
- (1977); ibid. 60, 473 (1977); T. Yamamori et al., Science 246, 1412 (1989); S. Saadat, M. Sendtner, H. Rohrer, J. Cell Biol. 108, 1807 (1989)
- M. Rao, S. Tyrrell, S. Landis, P. Patterson, Dev. Biol. 150, 2811 (1992).
- S. Landis, Trends Neurosci. 13, 344 (1990).
- M. Rao and S. Landis, J. Neurobiol. 24, 215 5 (1993). 6. S. Landis and D. Keefe, Dev. Biol. 98, 349 (1983).
- G. Leblanc and S. Landis, J. Neurosci. 6, 260 (1986).
- S. Landis, R. Siegel, M. Schwab, Dev. Biol. 126, 129 (1988).
- L. Stevens and S. Landis, ibid. 123, 179 (1987) 10. R. Schotzinger and S. Landis, Nature 335, 637
- (1988). Neuron 5, 91 (1990). 11
- M. Rao and S. Landis, *ibid.*, p. 899.
   M. Rao, P. Patterson, S. Landis, *Development* 116, 731 (1992).
- 14. H. Rohrer, ibid. 114, 689 (1992).
- 15. S. Tresser, M. Rao, S. Landis, Soc. Neurosci.
- Abstr. 18, 1475 (1992). 16. P. Walicke and P. Patterson, J. Neurosci. 1, 333 (1981).
- 17. M. Yodlowski, J. Fredieu, S. Landis, ibid. 4, 1535 (1984).
- 18. B. Habecker, unpublished observations.
- D. Clegg, T. Large, S. Bodary, L. Reichardt, *Dev. Biol.* 134, 30 (1989); H. Rohrer, R. Heumann, H. 19 Thoenen, ibid. 128, 240 (1988).
- 20. M. Grant and S. Landis, J. Neurosci., in press. 21. Sympathetic and sensory neuron cultures were prepared essentially as described by E. Hawrot and P. Patterson [Methods Enzymol. 58, 574 (1979)] and by Rao and Landis (12), except that dorsal root ganglia were incubated for only 40 min in dispase and collagenase. Non-neuronal cells were removed by preplating, and neurons were grown in 10 µM arabinosylcytosine for the first 2

days. Neurons were plated with 25,000 to 30,000 cells per well in 24-well plates for transfilter exper iments and 3000 to 4000 cells per well in 96-well plates for other experiments. Primary rat sweat aland cultures were established by explanting tissue from hind footpads of P7 to P9 rats. Pads were treated with dispase and collagenase for 75 min at 37°C before gland-containing regions were explanted and cells were grown to near confluence in serum-free medium [D. Gruenert, C. Bas-baum, J. Widdicombe, In Vitro Cell. Dev. Biol. 26, 411 (1990); G. Collie, M. Buchwald, P. Harper, J. Riordan, ibid. 21, 257 (1985)]. Gland cells were added to neurons after 2 days,  $4 \times 10^5$  cells per well in 96-well plates,  $4 \times 10^6$  cells per well in 24-well plates or in filter inserts.

- 22. Cholinergic function was determined by the presence of choline acetyltransferase (ChAT) activity, assayed by the method of F. Fonnum [Biochem. J 115, 465 (1969)] as modified by Rao and Landis (12). Data were analyzed by analysis of variance with Statview II (Abacus Concepts, Berkeley, CA).
- 23. Conditioned medium was collected from day 5. concentrated through a 10-kD Centricon filter for 90 min at 3000 rpm, diluted in medium, and sterilized through a 0.22-µm filter
- 24 RNA was isolated by the method of P. Chomczynski and N. Sacchi [Anal. Biochem. 162, 156 (1987)]. Samples were reverse-transcribed with the Promega cDNA synthesis kit and amplified by polymerase chain reaction through 35 cycles (1 min each at 94°, 52°, and 72°Č) with primers encoding subtype-specific regions of receptor proteins:  $\alpha_1(+)$ TCTAGGCGCGTGCGCTTACA, (-)GCCAGGTCGGTCGTGGTGTC (bases 1373 to 1393 and 1713 to 1733) [M. Voight, J. Kispert, H. Chin, *Nucleic Acids Res.* **18**, 1053 (1990)];  $\alpha_2$  (+)CCCAAGCCTCCAAGAGAGAGAA, (-)CCACTG-GCAACTCCCACATT (bases 1133 to 1153 and

1417 to 1437) [D. Zeng et al., Proc. Natl. Acad. Sci. U.S.A. 87, 3102 (1990)];  $\beta_1$  (+)GCCCA-GAAACAGGTGAAGAA, (-)CCTTGGACTCG-GAAACAGGTGAAGAA, (-)CCTTGGACTCG-GAGGAGAAG (bases 760 to 780, and 1376 to 1396) [C. Machida et al., J. Biol. Chem. 265. 12960 (1990)];  $\beta_2$  (+)AGAGCACAAAGCCCTCA-AGA, (-)AGCCGTTCCCATAGGTTTTC (bases 804 to 824 and 1044 to 1064) [P. Buckland et al., Nucleic Acids Res. 18, 682 (1990)]. Hybridization was carried out in Quickhyb (Stratagene) with  $^{32}\text{P-labeled}$  oligonucleotides [ $\alpha_1$ , bases 1442 to 1472;  $\beta_1$ , bases 930 to 960 (cross-hybridizes with  $\beta_2$ )] that were washed in 0.1× standard saline citrate and 0.2% SDS at 45°C and exposed onto film overnight.

- Rats at P10 were perfused with phosphate-buff-25. ered saline, and 10- $\mu m$  footpad sections were mounted on gelatin-coated slides. For  $\alpha_1$ , sections were incubated with 50 pM (2-{B(4-hydroxy-3-[1251]iodophenyl)-ethylaminomethyl}-tetralone) with or without 100 nM prazosin as described [H. Glossmann, F. Lübbecke, P. Presek, *Eur. J. Pharmacol.* **75**, 149 (1981)]. For  $\beta$ , sections were incubated with 50 pM <sup>125</sup>I-labeled pindolol with or without 10 µM propranolol [V. Arango et al., Brain Res. 516, 113 (1990)]. Autoradiography and receptor quantitation were completed as described by M. Grant, S. Landis, and R. Siegel [J. Neurosci. 11, 3763 (1991)]. Autoradiograms were exposed for 2 days at 4°C.
- 26. We thank P. Ernsburger, L. Collins, and R. Siegel for advice concerning binding and autoradiography; N. Malec for technical assistance with binding and quantitation; and S. Tresser for replicating the transwell experiment. Supported by an NIH grant to S.C.L. (NS-023678) and by an NIH postdoctoral fellowship award to B.A.H

27 January 1994; accepted 21 April 1994

## Digenic Retinitis Pigmentosa Due to Mutations at the Unlinked Peripherin/RDS and ROM1 Loci

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In spite of recent advances in identifying genes causing monogenic human disease, very little is known about the genes involved in polygenic disease. Three families were identified with mutations in the unlinked photoreceptor-specific genes ROM1 and peripherin/RDS, in which only double heterozygotes develop retinitis pigmentosa (RP). These findings indicate that the allelic and nonallelic heterogeneity known to be a feature of monogenic RP is complicated further by interactions between unlinked mutations causing digenic RP. Recognition of the inheritance pattern exemplified by these three families might facilitate the identification of other examples of digenic inheritance in human disease.

**R**etinitis pigmentosa (RP) is the name given to a set of hereditary human diseases that cause blindness resulting from degeneration of rod and cone photoreceptors in the retina. Patients typically develop night blindness in adolescence, lose midperiph-

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eral vision in young adulthood, and are blind by middle age. In the United States alone 50,000 to 100,000 people are afflicted with RP. Oral vitamin A supplementation has been reported to slow but not stop the course of the disease (1). RP exhibits genetic heterogeneity and can be transmitted as an autosomal dominant, autosomal recessive, or X-linked trait. Most cases are considered to be monogenic; that is, in any given family, only one responsible locus is thought to be defective. Although polygenic inheritance could explain the variable expressivity seen in some pedigrees (2), no specific combinations of genes have been identified that cause or modify this disease.

We identified three families with RP

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that segregated the missense mutation Leu185Pro in the human peripherin/RDS gene (Fig. 1). One of these families, #6935, was reported earlier by us as an example of autosomal dominant RP caused by this mutation (3). The three families collectively have the following unusual features for a dominant disease: (i) In each family, the disease originates in the offspring from a recent or an ancestral mating of unaffected individuals. (ii) Affected individuals as a group transmit the disease to fewer than 50% of their offspring [11 affected offspring out of 45 offspring from affected individuals ( $\chi^2 = 11.8$ ; df = 1; P < 0.001)]. (iii) Whereas affected individuals invariably carry the Leu185Pro allele, many asymptomatic individuals are also carriers. We did not detect any other pathogenic mutation in either allele of the affected patients after screening the entire proteincoding region of the peripherin/RDS gene. A possible explanation for the variable expression in these families is that the retinal degeneration associated with the Leu185Pro allele required an additional gene defect at another locus. We found evidence strongly supporting this hypothesis when we screened these families for mutations in the ROM1 gene.

The ROM1 gene is similar to the peripherin/RDS gene in its genomic structure, its photoreceptor-specific expression, and in the primary and probably secondary structure of its protein product (4). The two genes are not linked: Peripherin/RDS has been assigned to chromosome 6p and ROM1 to 11q (5). The encoded proteins form homodimers that interact noncovalently with each other. They have been hypothesized to play an important role at the rim region of photoreceptor outer segment disc membranes (6).

Starting with the human complementary DNA (cDNA) sequence (6), we used the polymerase chain reaction (PCR) to amplify and sequence two introns of the ROM1 gene. On the basis of this genomic

sequence, we divided the gene into eight overlapping regions that could be amplified by PCR. We used the technique called single-strand conformation polymorphism (SSCP) (7) to screen affected patients from these three families for mutations in the ROM1 gene. Two distinct 1-base pair (bp) insertions in exon 1 were found (Fig. 2). One mutation, termed Gly80(1-bp ins), was found in families #6285 and #6935. The other mutation, Leu114(1-bp ins), was found in family #5509. Both mutations are frameshifts early in the coding region resulting in a downstream premature stop at codon 131. As such, they probably do not encode a functional protein and therefore are likely to be null alleles.

Analysis of the segregation of the peripherin/RDS and ROM1 alleles revealed that all affected individuals carry both the peripherin/RDS (Leu185Pro) allele and a ROM1 null allele heterozygously (Fig. 1). The heterozygous carriers of either the peripherin/RDS (Leu185Pro) allele alone or a



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**Fig. 1.** Retinitis pigmentosa pedigrees #6285, #6935, and #5509 segregating the peripherin/*RDS* (Leu185Pro) allele. Filled symbols indicate individuals with RP. Probands are indicated by arrows. For all family members who contributed a blood sample for DNA analysis, the genotypes at the peripherin/*RDS* and *ROM1* loci are underneath the respective symbols with peripherin/*RDS* alleles above *ROM1* alleles (24). In all three families, the allele designation "rds" denotes the Leu185Pro allele; the designation "+" denotes a wild-type allele at the peripherin/*RDS* or *ROM1* loci. In families #6285 and #6935, the designation "rom" denotes the *ROM1* allele Gly80(1-bp ins); in family #5509, the designation "rom" denotes the *ROM1* allele Leu114(1-bp ins). In a previous report describing family #6935, we found individual IV-1 to carry the peripherin/*RDS* mutation Leu185Pro (*3*); subsequent to that publication, the patient agreed to be examined and was found not to have RP. Analysis of a second blood sample revealed that he did not carry Leu185Pro and that the previous erroneous result, corrected in this figure, was due to a mix-up of DNA samples in our laboratory. In addition, the arrangement of the siblings of IV-1 has been revised so that they are now in their birth order, left to right.

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ROM1 null allele alone were asymptomatic and had no funduscopic signs of RP (no intraretinal bone-spicule pigmentation, optic disc pallor, or vascular attenuation). Double heterozygotes could be clearly distinguished from single heterozygotes by means of the electroretinogram (ERG). Individuals carrying both a peripherin/RDS and a ROM1 mutation had severely reduced ERG amplitudes typical of RP (8, 9), whereas carriers of either a peripherin/RDS or a ROM1 defect alone had normal or slightly below normal amplitudes (Table 1). The distinction is most marked for the rod-plus-cone responses to white flashes of light at 0.5 Hz, where the only asymptomatic carriers who fall substantially below the normal range are elderly. A decrease in ERG amplitude with age is well documented (10), and it is possible that some of the decrease in the ERG amplitudes found among these elderly carriers is due to an age effect. However, the borderline ERG amplitudes found in many of the asymptomatic, single gene carriers are comparable with those found in some heterozygous carriers of recessive RP alleles in the rhodopsin (11) or the rod phosphodiesterase- $\beta$  subunit genes (12), suggesting that the peripherin/ RDS (Leu185Pro) allele and the ROM1 null alleles are in themselves recessive.

These findings prompted us to evaluate the peripherin/RDS gene in additional patients with autosomal recessive RP and to evaluate ROM1 in additional patients with autosomal recessive or dominant RP. A screen in which the SSCP technique was used to examine 132 unrelated cases of autosomal recessive RP for mutations in the peripherin/RDS gene was negative and, in particular, did not identify additional cases with the Leu185Pro mutation. Separate screens of 190 unrelated cases of autosomal recessive RP and 185 unrelated cases of autosomal dominant RP revealed no additional ROM1 mutations that were clearly null. Because these screens were unfruitful, we do not know what phenotype, if any, would be conferred by homozygosity for either the peripherin/RDS (Leu185Pro) allele or one of the ROM1 null alleles. However, in families #5509, #6285, and #6935, it is likely that we are observing the noncomplementation of two unlinked recessive alleles because (i) heterozygotes for either one alone have ERGs similar to recessive RP gene carriers; (ii) combined heterozygosity for both produces RP; and (iii) the two protein products form an intermolecular complex, and this type of interaction at the protein level is a feature of genes in other species in which recessive alleles do not complement each other (13). An alternative formal possibility is that these mutations are in themselves not recessive (that is, homozygosity for any of



**Fig. 2.** (A) Sequence of the *ROM1* gene around codon 80 in individual IV-19 of pedigree #6285, showing heterozygosity for a 1-bp insertion of a G, highlighted with brackets (*25*). (B) Sequence of the *ROM1* gene around codon 114 in individual I-2 of pedigree #5509, showing heterozygosity for a 1-bp insertion of a G (*25*). Each sequence is adjacent to the sequence of a control individual for comparison. These mutations alter the specificity of the codons immediately downstream (designated by blanked letters) in the mutant alleles; both change codon 131 to a premature stop. The amplified DNA samples from the respective patients were also sequenced in the antisense direction with confirmatory results.

these alleles might result in no observable phenotype) and that only the combination of the two unlinked heterozygous mutations

is pathogenic. The term "digenic retinitis pigmentosa" encompasses both possible genetic mechanisms.

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**Table 1.** Full-field ERGs recorded from affected and unaffected members from the three families. Listed values are the average of both eyes at the initial visit. ERG amplitudes elicited in response to dim blue flashes at 0.5 Hz are a measure of rod function, responses to white flashes at 0.5 Hz are a measure of rod-plus-cone function, and responses to white flashes at 30 Hz are a measure of cone function (*9, 23*). Implicit time refers to the interval between a flash of light and the peak of the resulting cornea-positive wave; substantial increases in implicit time are characteristic of RP (*9*). The normal range (nr) of values for each measurement is given in parenthesis. ND, not detectable; NT, not tested. Our procedure for recording ERGs has been previously reported (*9, 23*).

Pedi- gree	Mem- ber	Age	ERG amplitude (µV)			Implicit time (ms)
			Blue 0.5 Hz (nr 100–275)	White 0.5 Hz (nr 350–700)	White 30 Hz (nr 50–125)	White 30 Hz (nr 25–32)
RDS, Leu185Pro/+; ROM1, +/+						
5509	I-1	68	41	235	24	33
6285	III-18	63	50	244	44	31
6285	IV-34	39	62	347	59	33
6285	IV-37	38	94	353	68	33
5509	II-7	35	100	382	53	33
6285	IV-42	31	115	388	70	33
5509	III-2	14	126	412	53	29
5509	III-3	11	171	512	76	29
RDS, +/+; ROM1, Gly80(1-bp ins)/+						
6935	IV-10	38	147	635	91	29
6285	V-2	25	129	418	52	32
6285	V-5	27	100	371	62	31
RDS, +/+; ROM1, Leu114(1-bp ins)/+						
5509	I-2	68	50	353	32	33
5509	II-3	35	124	388	68	31
5509	II-9	24	82	399	50	32
RDS, Leu185Pro/+; ROM1, Gly80(1-bp ins)/+						
6935	III-9*	52	ND	<1.0	1.2	45
6285	IV-15*	51	NT	1.2	0.5	48
6285	IV-19*	37	NT	8.3	5.6	47
6935	IV-13*	18	NT	35.2	19.5	45
RDS, Leu185Pro/+; ROM1, Leu114(1-bp ins)/+						
5509	II-1*	34	NT	21.6	 3.3	44
5509	II-6*	30	ND	35.3	17.6	44

\*Affected with RP.

Mutations other than Leu185Pro in the human peripherin/RDS gene have been observed to cosegregate with autosomal dominant RP (3, 14), retinitis punctata albescens (15), or certain hereditary macular dystrophies (16-18). In view of the patients with combined ROM1 and peripherin/RDS mutations described here, one might speculate that the range of phenotypes exhibited by the reported peripherin/RDS mutations might be due in part to contributory mutations in ROM1 or other unlinked genes. However, at least some reported mutations in peripherin/RDS, such as Pro219del (3), Pro216Leu (3), and Ser212Gly (19), perfectly cosegregate with RP in large families in which the disease transmission rate is convincingly closer to 50% than 25%. Furthermore, we used the SSCP technique to analyze affected individuals from families with Pro219del and Pro216Leu and found no ROM1 defects. Consequently, some peripherin/RDS gene defects seem to be dominant alleles not requiring mutations in ROM1 or other unlinked loci to produce a retinal degeneration phenotype. On the other hand, we note here that published pedigrees segregating the Cys118del allele (17) and possibly the Gly167Asp (16) allele fit a digenic inheritance pattern better than a monogenic one. In the pedigree with the Cys118del allele (17), for example, the disease appears to originate in the offspring of an ancestral mating of unaffected individuals, and of the 15 affected members or obligate carriers who procreated, the disease was transmitted to only 18 of 65 offspring. These latter pedigrees suggest that the requirement for a contributing unlinked mutation is not specific to the Leu185Pro allele of peripherin/ RDS. The property specific to the category of peripherin/RDS mutations that obligates another gene defect to cause disease remains obscure.

Retinitis pigmentosa is known to be genetically heterogeneous. Monogenic forms due to mutations in either the rhodopsin (20), peripherin/RDS (3, 14), or rod phosphodiesterase- $\beta$  subunit (12) genes collectively account for less than 25% of cases, and linkage studies have implicated unidentified loci on chromosomes 1q, 7p, 7q, 8, 11p, 11q, 14q, 16q, 19q, and Xp that

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collectively account for an unknown proportion of additional cases (21). If digenic inheritance in RP is common, it will complicate current approaches to identify genes responsible for this disease using the positional cloning approach. Linkage studies of recessive RP will be particularly complex, as provision must be made for the possibility of two unlinked loci causing disease in some families, as is illustrated in our pedigree #5509, which appears recessive at first glance. It is also possible that some pedigrees with dominant RP with reduced or incomplete penetrance (2) might have the fortuitous segregation of mutations at two unlinked, noncomplementing loci, in which case linkage studies that successfully identify a chromosomal region shared by all affected members would be incomplete. Candidate gene analyses similar to that performed in this study should be successful in identifying some of the other RP genes and in elucidating which combinations of them produce disease.

## **REFERENCES AND NOTES**

- 1. E. L. Berson *et al.*, Arch. Ophthalmol. **111**, 761 (1993).
- E. L. Berson and E. A. Simonoff, *ibid.* 97, 1286 (1979); M. Jay, A. C. Bird, A. N. Moore, B. Jay, *J. Med. Genet.* 29, 906 (1992).
- 3. K. Kajiwara *et al., Nature* **354**, 480 (1991).
- R. A. Bascom, K. Schappert, R. R. McInnes, *Hum. Mol. Genet.* 2, 385 (1993).
- G. H. Travis *et al.*, *Genomics* **10**, 733 (1991); R. A. Bascom *et al.*, *Am. J. Hum. Genet.* **51**, 1028 (1992).
- 6. R. A. Bascom et al., Neuron 8, 1171 (1992).
- M. Orita, H. Iwahana, H. Kanazawa, K. Hayashi, T. Sekiya, *Proc. Natl. Acad. Sci. U.S.A.* 86, 2766 (1989).
- 8. G. Karpe, Acta Ophthalmol. 23 (suppl. 24), 1 (1945).
- 9. E. L. Berson, Trans. Am. Acad. Ophthalmol. Otolaryngol. 81, 659 (1976).
- R. G. Weleber, Invest. Ophthalmol. Visual Sci. 20, 392 (1981).
- 11. P. J. Rosenfeld et al., Nature Genet. 1, 209 (1992). 12. M. E. McLaughlin, M. A. Sandberg, E. L. Berson,
- T. P. Dryja, *ibid.* **4**, 130 (1993).
- T. S. Hays, R. Deuring, B. Robertson, M. Prout, M. T. Fuller, *Mol. Cell. Biol.* 9, 875 (1989); T. Stearns and D. Botstein, *Genetics* 119, 249 (1988); M. Kusch and R. S. Edgar, *ibid.* 113, 621 (1986).
- 14. G. J. Farrar et al., Nature 354, 478 (1991).
  - K. Kajiwara, M. A. Sandberg, E. L. Berson, T. P. Dryja, *Nature Genet.* 3, 208 (1993).
  - 16. B. E. Nichols *et al.*, *ibid.*, p. 202.
  - 17. J. Wells *et al., ibid.*, p. 213. 18. R. G. Weleber, R. E. Carr, W. H. Murphey, V. C.
  - Sheffield, E. M. Stone, Arch. Ophthalmol. 111, 1531 (1993). 19. G. J. Farrar et al., Genomics 11, 870 (1991); G. J.
  - G. J. Farrar *et al.*, *Genomics* 11, 870 (1991); G. J.
     Farrar *et al.*, *Am. J. Hum. Genet.* 51 (suppl. A94), 363 (1992).
  - 20. T. P. Dryja, *Eye* **6**, 1 (1992); E. L. Berson, *Invest. Ophthalmol. Visual Sci.* **34**, 1659 (1993).
  - W. J. Kimberling *et al.*, *Genomics* 7, 245 (1990);
     R. A. Lewis, B. Otterud, D. Stauffer, J. M. Lalouel,
     M. Leppert, *ibid.*, p. 250; C. F. Inglehearn *et al.*,
     *Nature Genet.* 4, 51 (1993); S. A. Jordan *et al.*,
     *ibid.*, p. 54; S. H. Blanton *et al.*, *Genomics* 11,
     857 (1991); R. J. Smith *et al.*, *ibid.*, p. 988; J.
     Kaplan *et al.*, *ibid.*, p. 979; A. E. Kwitek-Black *et al.*,
     *Nature Genet.* 5, 392 (1993); M. Al-Maghtheh
     *et al.*, *Hum. Mol. Genet.* 3, 351 (1994); J. D.

Chen *et al.*, *Am. J. Hum. Genet.* **45**, 401 (1989). 22. K. Kajiwara, E. L. Berson, T. P. Dryja, unpublished data

- E. Reichel, A. M. Bruce, M. A. Sandberg, E. L. Berson, Am. J. Ophthalmol. 108, 540 (1989).
- 24. Analysis of the peripherin/RDS allele Leu185Pro was according to previously published methods (3). To identify mutations in *ROM1* and to study their cosegregation, we first sequenced the introns of the ROM1 gene. On the basis of the cDNA sequence of the human ROM1 gene (6), we synthesized pairs of oligonucleotide primers to amplify the two intron sequences by the polymer-ase chain reaction (PCR). Pairs of primers (ei-ther sense: 5'-CAGTGGGTCAGCAGCCGTTA-3' or sense: 5'-AGTTCCCTGTCCTGCCCCAG-3' and either antisense: 5'-AGGGACCCCATCAGT-CAGGT-3' or antisense: 5'-AGTCCCGGGTGG-GAGGAGGT-3') and one pair of primers (sense: 5'-TGGGTAGCATGCTGGCTGTC-3' and antisense: 5'-CCTCCAAGCCCCTCCAGTGC-3') successfully amplified intron 1 and intron 2, respectively. The amplified fragments were used as templates for direct sequencing [D. W. Yandell and T. P. Dryja, in Cold Spring Harbor Symposium Series, M.

Furth and M. Greaves, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989), vol. 7, pp. 223–2271 of both the sense and antisense strands with the above-mentioned primers and one additional primer (antisense: 5'-AGCCAAG-CATAATTCACATC-3') derived from the resulting intron 1 sequence. The human ROM1 locus was found to contain two introns at positions identical to those found by another group (4). Intron 1 has either 386 or 387 bp because of a polymorphism (one or two T's at position -26 or -27 from the 3' end of intron 1) (22). After obtaining intron se-quences, we divided the protein-coding sequences, we divided the protein-coding se-quence into eight regions to screen for mutations by SSCP (7). The patients who showed variant bands on SSCP gels were analyzed further by directly sequencing their genomic DNA. To study segregation of *ROM1* mutations with SSCP, we used a pair of primers (sense: 5'-AGTTCCCTGT-COTCOCAD 2' and anticense; 5'-AGTTCCCTGT-CCTGCCCCAG-3' and antisense: 5'-AGGGCCT-CCAGCGCCTC-3') encompassing codons 80 and 114 in exon 1 for PCR-mediated amplification. PCR was performed in 50  $\mu$ l of a solution containing 20 mM tris (pH 8.6), 50 mM KCI, bovine serum albumin (100 ng/ $\mu$ l), 0.5 mM MgCl<sub>2</sub>,

20 nM each deoxynucleotide triphosphate, genomic DNA (2 ng/µl), 10% dimethyl sulfoxide, and 0.5 units of Taq polymerase. The annealing temperature was 52°C, the polymerization temperature was 94°C. The amplified fragments were separated by electrophoresis through 9% polyacrylamide gels. Variant bands corresponding to the mutant *ROM1* alleles were easily distinguished from wild-type bands by this technique.

- type bands by this technique.
  25. Template DNA for direct sequencing was made by PCR with the primers sense: 5'-CAGTGGGT-CAGCAGCCGTTA-3' and antisense: 5'-AGGGC-CTCCAGCGCCTC-3'. PCR was performed as in (24). For sequencing, the sense primer 5'-AGT-TCCCTGTCCTGCCCCAG-3' was used.
- 26. We thank D. Lin, J. Morrow, J. Quinn, P. Rodriguez, M. Sandberg, and T. Skop for their assistance. This work was supported by grants from NIH (EY08683 and EY00169) and the RP Foundation Fighting Blindness, Baltimore, MD. K.K. is the recipient of a physician research fellowship from the Howard Hughes Medical Institute.

4 March 1994; accepted 27 April 1994