Retinitis Pigmentosa
The Friedenwald Lecture

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Retinitis pigmentosa (RP) has a prevalence of about 1 in 4000.[1-7] An estimated 1.5 million people are affected around the world. In the United States, based on a survey in Maine, the frequency of families by genetic type has been estimated to be 19% dominant, 19% recessive, 8% X-linked, 46% isolates, and 8% undetermined.[6] Molecular genetic techniques have revealed dominant forms on chromosomes 3, 6, and 8; a recessive form on 3; a recessive form with partial deafness called Usher's syndrome, type 2 on 1; and at least two X-linked forms on the short arm of the X-chromosome.[8-18] Thus, retinitis pigmentosa is a group of diseases caused by gene abnormalities on several chromosomes.

The symptoms and signs of retinitis pigmentosa are well known.[19-31] Affected patients typically report impaired adaptation, night blindness, and difficulty with mid peripheral visual field in adolescence. As the condition progresses, they develop a tendency to blue blindness, lose far peripheral field, and eventually lose central vision as well. Patients can have a normal fundus appearance in the early stages. In more advanced stages, signs include attenuated retinal vessels, intraretinal pigment, and waxy pallor of the optic discs. The intraretinal pigment is distributed circumferentially around the mid periphery in the zone where rods normally are at maximum concentration. Cataracts develop in most cases,[32] and some have cystoid macular edema.[33,34] Refractive errors, including myopia and astigmatism, are common.[35,36] Some patients become blind as early as age 30; the majority are legally blind by age 60 with a central visual field diameter < 20°.

Two rare forms of retinitis pigmentosa, associated with the Bassen-Kornzweig syndrome[36-42] and Refsum disease,[43-50] respectively, yielded to treatment once the biochemical abnormalities were understood. Patients with Bassen-Kornzweig syndrome cannot efficiently transport fat-soluble vitamins from the intestine to the plasma. Treatment of a patient with large doses of vitamin A at an early stage resulted in reversal of the electroretinogram (ERG) to normal within 24 hr.[40] Vitamin E also has been advocated to prevent progression of this retinal degeneration.[41,42] Patients with Refsum disease have an elevated serum phytanic acid resulting from a deficiency of phytanic acid oxidase. This fatty acid accumulates in the retinal pigment epithelium, leading to photoreceptor cell degeneration. Treatment with a low-phytol, low-phytanic acid diet has resulted in the lowering of serum phytanic acid and stabilization of retinal function.[48,50]

ERG Testing as an Aid in Early Detection of RP

In the mid 1960s, some uncertainty existed regarding differentiating the early stages of progressive forms of retinitis pigmentosa versus the early stages of self-limited or stationary retinal disease. We evaluated asymptomatic offspring of patients with known retinitis pigmentosa.[51-56] Figure 1 illustrates ERGs from four affected children with the different genetic types. All have rod responses to blue light that are either nondetectable or reduced in amplitude with delayed b-wave implicit times, as indicated by the horizontal arrows. Mixed cone-rod responses to single 0.5 Hz flashes of white light are reduced with subnormal a-waves, indicating the early photoreceptor involvement. Cone responses to 30 Hz white flicker are normal or reduced and usually are so delayed with respect to implicit time that a phase shift occurs between the

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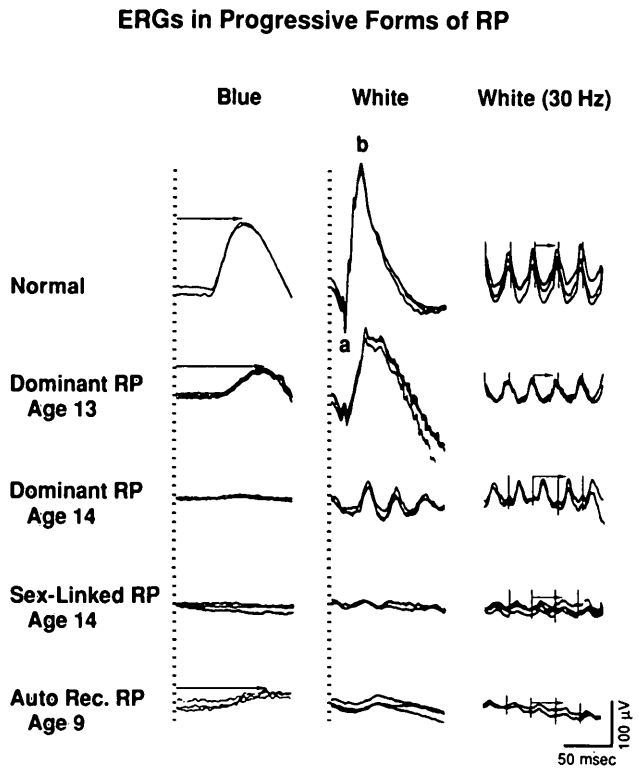


FIGURE 1. ERG responses from a normal subject and from four patients (ages 13, 14, 14, and 9) with retinitis pigmentosa. Stimulus onset is vertical hatched lines for columns 1 and 2 and vertical shock artifacts for column 3. Rod b-wave implicit times in column 1 and cone implicit times in column 3 are designated with arrows. Calibration symbol (lower right corner) signifies 50 msec horizontally and 100 μV vertically for all tracings. Under these test conditions, normal amplitudes are ≥100 μV (left column), ≥350 μV (middle column), and ≥50 μV (right column). Normal rod implicit time is ≤108 msec and normal cone implicit time is ≤32 msec. (From reference 56.)

stimulus artifacts—designated by the short vertical lines—and the corresponding response peaks. These reduced and delayed ERGs can be detected in some cases years to a decade before symptoms develop or diagnostic changes are visible with the ophthalmoscope.

Figure 2 shows reduced ERGs with normal implicit times in self-limited or stationary retinal disease. For example, a father and son with self-limited, sector retinitis pigmentosa have reduced rod responses with b-wave implicit times in the normal range, defined by the vertical bar. Cone responses to 30 Hz flicker are reduced but not shifted in phase. The father and son have virtually identical responses, even though the difference in their ages is about 30 yr.[57] Other nonprogressive conditions, such as stationary night blindness with myopia[58] or Oguchi's disease,[56] also have cone responses that are normal in implicit time.

That delays in implicit times identify those with progressive retinitis pigmentosa has been substantiated by following the same patients over time.[59,60] For example, as shown in Figure 3 in a family tested over a 10 yr interval, all affected members show declines in amplitudes. The bottom row of tracings shows a patient with detectable 30 Hz cone responses at age 17, but nondetectable responses at age 26. That is, the responses have declined to less than 10 μV and no longer can be visualized without computer averaging. Figure 4 illustrates that ERGs that would be nondetectable without computer averaging are easily visualized with computer averaging. Progression can be seen over a 2 yr interval in an adult with responses below 10 μV. Computer averaging with narrow bandpass filtering has extended the range of detectability of 30 and 42 Hz cone responses to well under 1 μV, permitting us to monitor the course of the disease objectively in most patients. In most instances, patients are legally blind when their ERG amplitudes are below five one-hundredths of 1 μV.[60,61]

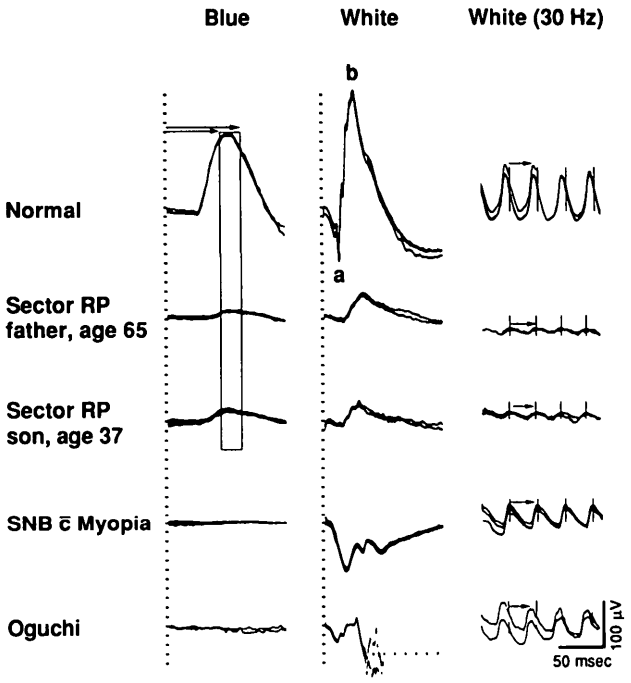


FIGURE 2. ERG responses from a normal subject and from four patients with sector or stationary retinal disease. Horizontal arrows (column 1) designate range of normal rod b-wave implicit times, and vertical bar defining this range (mean ± 2 SD) has been extended through responses of patients with sector retinitis pigmentosa. Responses (middle column) from patient with Oguchi's disease are interrupted by reflex blinking, so latter part cannot be illustrated. Cone implicit times in column 3 are designated with arrows. (From reference 56.)

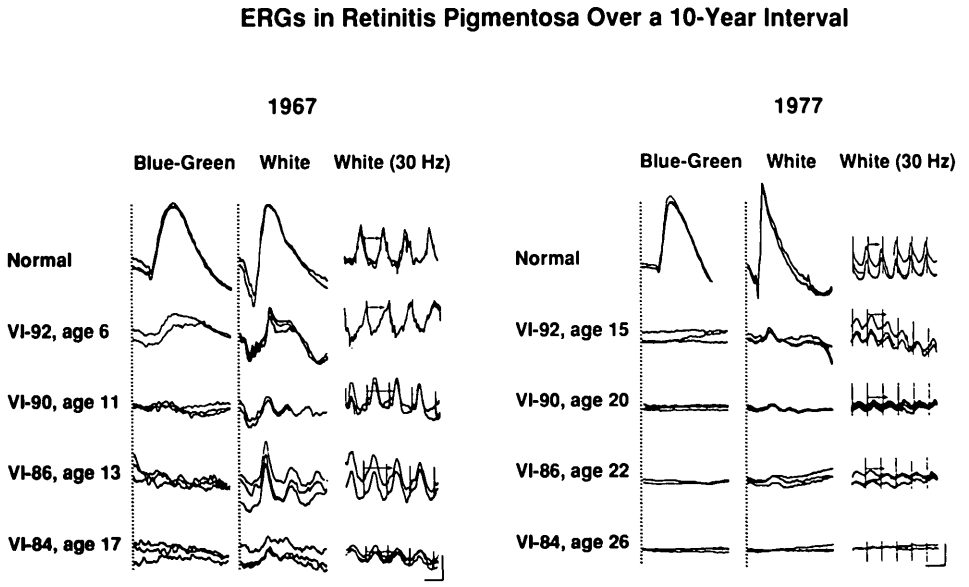


FIGURE 3. ERG responses recorded in 1967 and 1977 from a normal subject and from four affected members of a family with a dominant form of retinitis pigmentosa transmitted over six generations. Pedigree number and age at time of testing are indicated for each patient. One to three responses to same stimulus are represented. Calibration symbol for 1967 responses designates 50 msec horizontally for columns 1 and 2 and 25 msec for column 3 and 50 μV vertically for column 1 and 100 μV vertically for columns 2 and 3. Calibration symbol for 1977 responses designates 60 msec horizontally and 100 μV vertically for all tracings. (From reference 59.)

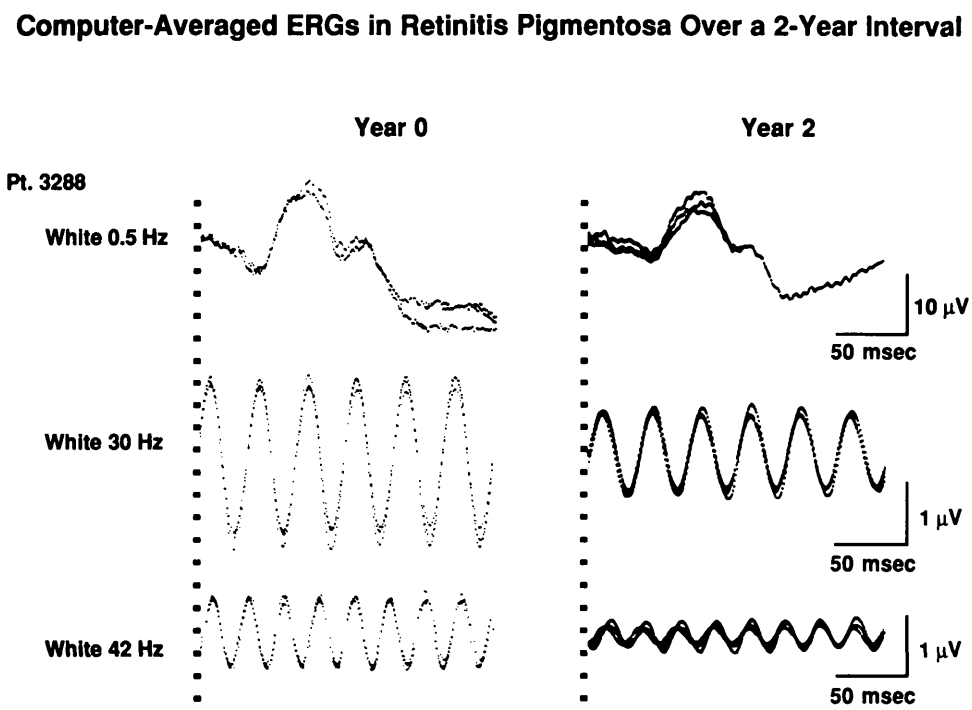


FIGURE 4. Computer-averaged full-field ERGs from a 26-yr-old man with X-linked retinitis pigmentosa in response to 10 μsec flashes of white light presented at 0.5 Hz (n = 64), 30 Hz (n = 256), and 42 Hz (n = 256) at baseline (year 0) and at 2 yr follow-up (year 2). Three consecutive response averages are superimposed in each case. The vertical hatched lines denote flash onset for the 0.5 Hz condition and the onset of one of a train of flashes for the 30 Hz and 42 Hz conditions. (From reference 60.)

The ERG has proved useful not only in identifying which patients are affected with retinitis pigmentosa but also in determining which relatives of affected patients are normal. The percentages of normal and abnormal ERGs among siblings, ages 6 to 20, in families with dominant or recessive disease correspond respectively with the percentages that would be predicted from the Mendelian laws of inheritance governing transmission of these diseases. Predicted percentages would be 50% normal and 50% affected among siblings in families with dominant disease, and 75% normal and 25% affected among siblings in families with autosomal recessive disease. In a study where all 11 siblings, ages 6 to 20, from six families with autosomal dominant retinitis pigmentosa were evaluated with ERG testing, 5 of 11, or 45.5%, were abnormal. When all 49 siblings from 18 families with autosomal recessive retinitis pigmentosa were evaluated, 11 of 49, or 25.6%, were abnormal.[62] Stated another way, the ERG results follow Mendel's laws. We have concluded that patients 6 yr old and over in families with retinitis pigmentosa with normal ERGs would not be expected to develop retinitis pigmentosa later.[50,62] This ability to distinguish affected from normal siblings has enabled us to provide long-term visual prognoses and also has had implications in our molecular genetic studies.

ERG testing also can help detect most female carriers of X-linked retinitis pigmentosa (Fig. 5).[63,64] ERGs from obligate carriers are reduced in amplitude, delayed in implicit time, or both.[63] Daughters of obligate carriers, who have a 50% chance of inheriting the carrier state, show normal responses, as seen in patient 3, or abnormal responses comparable to those of obligate carriers. The abnormal ERGs from X-linked carriers contrast with the normal responses typically seen under the same test conditions in female carriers of autosomal recessive disease.[63] The separation of X-linked from recessive retinitis pigmentosa has practical application in providing visual prognoses, because males with X-linked retinitis pigmentosa usually are legally blind by ages 30 to 45, whereas males with autosomal recessive retinitis pigmentosa usually are legally blind by ages 45 to 60.

Natural Course of RP

From 1979 to 1983, we conducted a prospective study on 94 randomly selected patients, ages 6 to 49, from separate families from across the United States representing the different genetic types in approximately equal numbers.[60] Entry requirements included visual acuity of 20/100 or better, visual field diameter of 8° or larger, and fundus findings of retinitis pigmentosa. We evaluated visual acuity, kinetic visual fields on the Goldmann perimeter with a V-4_e white test light, final dark-adapted thresholds to an 11° white test light, ERG amplitudes, and fundus appearance annually. Data on mean full-field ERG amplitudes for those with detectable responses are summarized in Table 1. In this population, with an average age of 24 yr at the beginning of this study, the mean 0.5 Hz response amplitude declined from 11.3 μV at baseline or year 0 to 6.7 μV at year 3. The mean 30 Hz amplitude de-

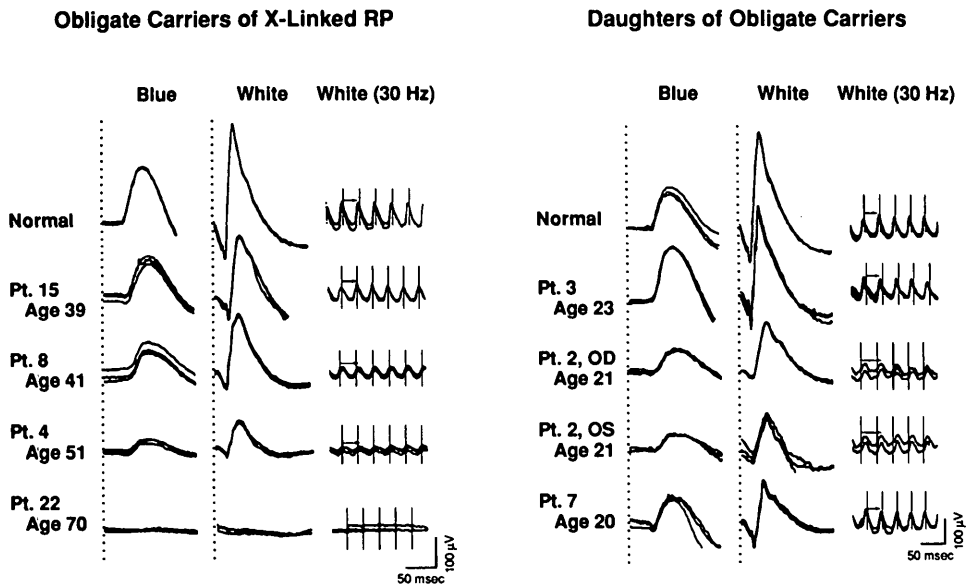


FIGURE 5. ERG responses from a normal subject and from four obligate carriers of X-linked retinitis pigmentosa on the left and from a normal subject and three daughters of obligate carriers of X-linked retinitis pigmentosa on the right. Patient 2 had reduced responses in both eyes but intraretinal pigment only in the left eye. (From reference 63.)

TABLE 1. Mean Change in Visual Function Over 3 Years in Study Population With RP

Test	*n*	*Yr 0 GM*	*Yr 1 GM*	*Yr 2 GM*	*Yr 3 GM*	*Yr 0–3 P*	*% Change (Annual)*
Visual acuity	91	20/39	20/45	20/44	20/40	NS	1.2
Visual field area, deg^2 * (diameter)	90	3463 (66.5°)	3395 (65.6°)	3197 (63.8°)	3011 (61.9°)	.038	4.6
Foveal ERG†	67	0.15	0.16	0.15	0.13	.009	5.2
Full-field ERG							
0.5 Hz‡	68	11.34	9.46	7.89	6.72	<.001	16.0
30 Hz§	76	2.16	1.76	1.28	1.17	<.001	18.5
37 or 42 Hz‖	75	0.92	0.76	0.57	0.52	<.001	17.1

Lower norms: *11,399 to V-4$_e$ (equals 120° circular diameter); †0.18μV; ‡350 μV; §50 μV; ‖44 μV.
GM, geometric mean. NS, not significant.
(Modified from reference 60.)

clined from 2.1 μV at year 0 to 1.1 μV at year 3. These changes from year 0 to year 3 are highly significant. These data also reveal the orderly, apparently exponential decline in retinal function that occurs in a group of retinitis pigmentosa patients, a decline that can be monitored with computer averaged and narrow-bandpass filtered responses.

Based on a threshold-of-change analysis, we found that ERGs declined significantly in 77% of patients with detectable responses at baseline. These patients lost on average 16 to 18.5% of remaining full-field ERG amplitude per year and 5.2% of remaining foveal cone ERG amplitude per year, indicating that loss of retinal function was primarily extrafoveal in these patients. Patients lost, on average, 4.6% of remaining field area per year, whereas visual acuity and dark adaptation thresholds remained relatively stable. Intraretinal pigment increased in 54% of those for whom we could make comparisons over a 3 yr interval, suggesting that this method of following the condition is not as sensitive as full-field ERG testing.[60]

Some Observations on Remaining Photoreceptors in RP

As a complement to studies on the natural course, autopsy eyes have been evaluated to gain more information about pathogenesis.[65–76] Figure 6 shows remaining cone photoreceptors in a 56-yr-old woman with recessive retinitis pigmentosa (top) and remaining rod photoreceptors in a 24-yr-old man with X-linked retinitis pigmentosa (bottom). Remaining cones and rods have shortened outer segments but retain intact cell bodies. Phagosomes sometimes are seen in the retinal pigment epithelium.

To determine how well such remaining photore-

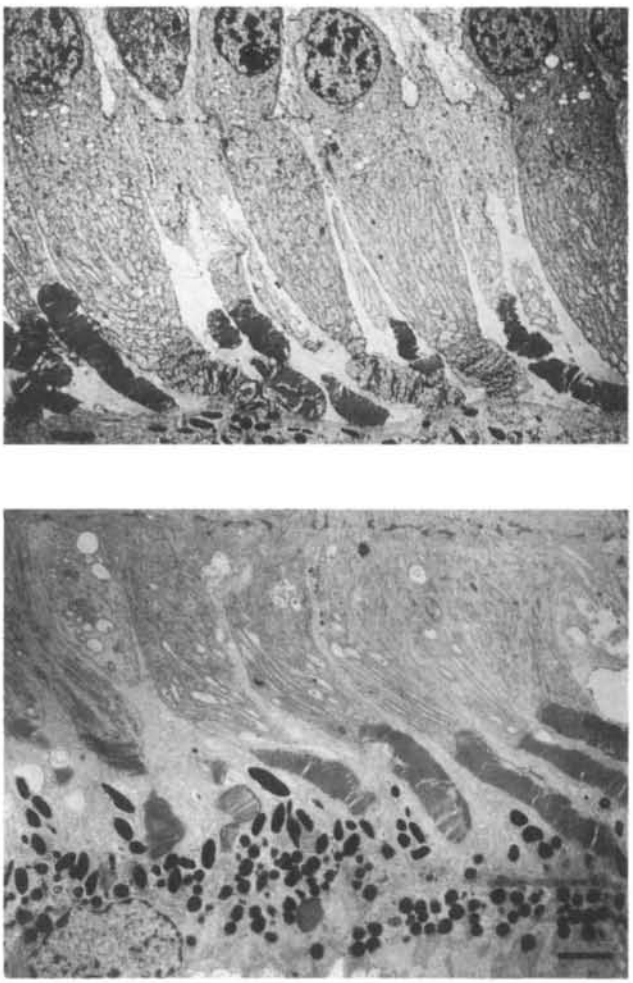


FIGURE 6. Electron micrographs from the macula of a 56-yr-old woman with recessive retinitis pigmentosa with partial deafness (top) and from the periphery of a 26-yr-old man with X-linked retinitis pigmentosa (bottom). (Bars = 3 μm.)

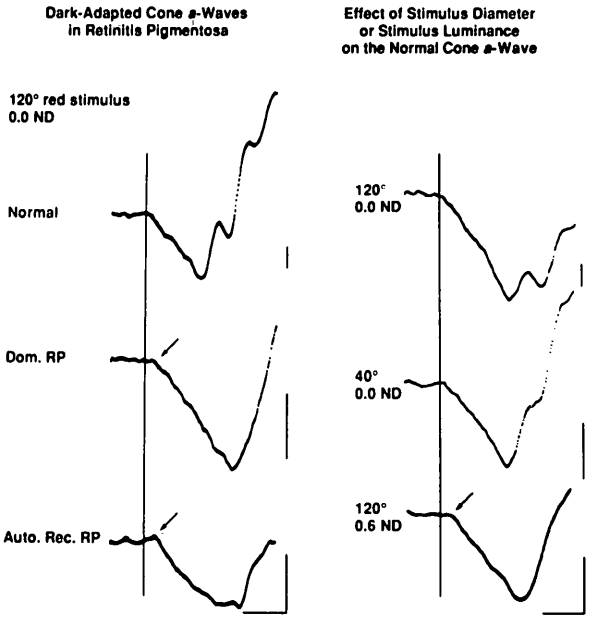


FIGURE 7. Computer-averaged cone ERG responses to a 10 μsec xenon flash (3.4 log foot-lamberts) attenuated with a Kodak Wratten 26 (red) filter and presented at 2 msec intervals from a normal subject, a patient with autosomal dominant RP, and a patient with autosomal recessive RP in the left column, and from normal subjects in the right column. Tracings begin at flash onset. Oblique arrows (left column) show delayed a-wave latencies for patients with RP compared with the normal; oblique arrow (right column) shows delayed a-wave latency for a normal subject produced by attenuation of stimulus luminance by a 0.6 log unit neutral density filter. Calibration bars vertically designate 5 μV and horizontally 10 msec. (Modified from reference 77.)

ceptors function, we recorded the cone ERG a-wave to a 120° diameter red light under dark-adapted conditions and measured the time interval between stimulus flash onset and the onset of the a-wave, or so-called a-wave latency (Fig. 7).[77] This test stimulus elicited no detectable response from a rod monochromat. A vertical line noting the time of onset of the a-wave in a normal subject has been extended through the patients' responses (Fig. 7, left column). A-wave onset in these patients is delayed, as noted by each oblique arrow. This delay can be explained by reduced absorption of light because of fewer cones or because remaining cones have shortened outer segments, or some combination of these two. In normal subjects (Fig. 7, right column), reduction of the stimulus diameter from 120° to 40°, as a simulation of reduced numbers of photoreceptors, resulted in reduced amplitudes but did not produce the delayed a-wave latencies seen in the patients. In contrast, reduction of stimulus intensity by 0.6 log neutral density units, as a simulation of reduced absorption of light by photoreceptors, did result in prolonged a-wave latencies like those seen in the patients. Regarding rod function (Fig. 8), Ripps showed that psychophysical rod threshold elevations can be correlated with rhodopsin densities as measured by fundus reflectometry in a group of patients with retinitis pigmentosa.[78] These data, taken together, suggest that remaining cone and rod photoreceptors in many patients with retinitis pigmentosa function normally for their numbers and for their amounts of remaining visual pigment, and lend support to the idea that these photoreceptors can be rescued.

Rhodopsin Gene Abnormalities in Autosomal Dominant RP

In the mid 1980s, we began to investigate the molecular genetic basis of retinitis pigmentosa as a way to determine the underlying biochemical causes. We decided to search for abnormalities in genes known to encode for proteins critical for photoreceptor cell function and viability. This has been called the "candidate gene" approach, and some of the candidate proteins, particularly those involved in the phototransduction cascade, are schematically represented in Figure 9.[79-86] The rhodopsin gene mapped to chromosome 3q,[87,88] and we were further encouraged to study this candidate gene after a report by Humphries and coworkers of linkage of an anonymous probe on 3q with dominant retinitis pigmentosa in a family in Ireland.[8]

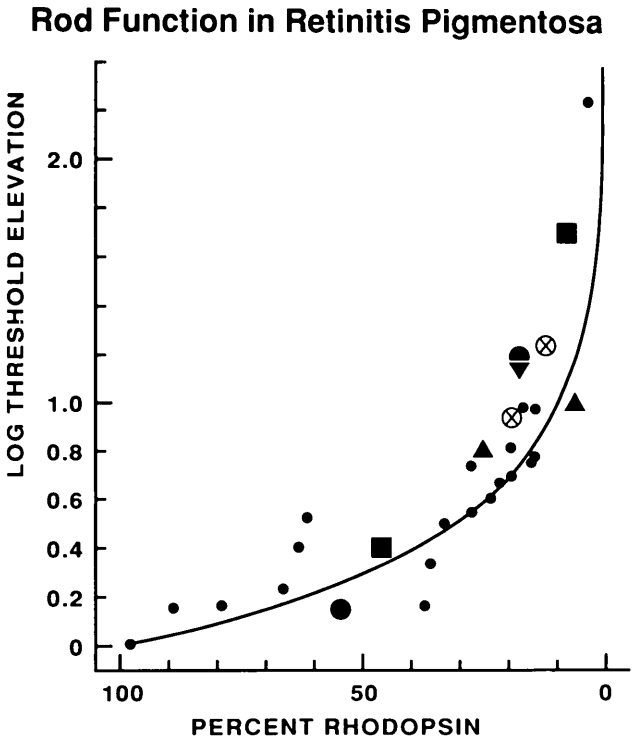


FIGURE 8. Log of absolute (dark-adapted) threshold plotted as a function of the percent rhodopsin measured at various retinal loci in patients with retinitis pigmentosa. Different symbols represent data derived from individual patients. (From reference 78.)

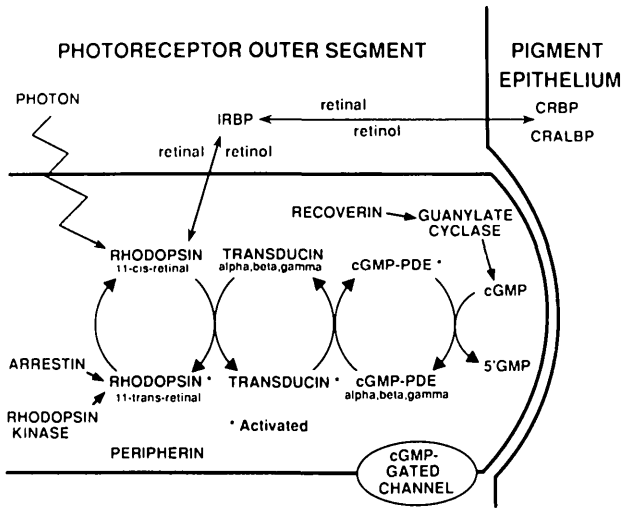


FIGURE 9. Schematic representation of some proteins normally found in the rod photoreceptor outer segment, interphotoreceptor matrix, and retinal pigment epithelium. Genes encoding these proteins are considered "candidate genes," because mutations in them could result in compromise of the phototransduction cascade or the mechanism by which vitamin A is transported between the photoreceptors and the pigment epithelium with consequent photoreceptor cell degeneration. IRBP, interphotoreceptor retinoid binding protein. CRBP, cellular retinol binding protein. CRALBP, cellular retinal binding protein. PDE, phosphodiesterase. cGMP, cyclic guanosine monophosphate.

As Nathans and his colleagues have shown, the rhodopsin gene is about 7000 bases in length and contains five exons, or coding regions.[79] The five exons are translated by messenger RNA into the rhodopsin protein, which consists of 348 amino acids identified consecutively from the amino to the carboxy terminus using a standard single-letter code (Fig. 10, top). For example, methionine, represented by "M," is at position 1, and alanine, represented by "A," is at position 348. Each molecule traverses the rod outer segment membrane seven times and has four loops facing the cytoplasm and three loops facing the intradiscal space. Vitamin A—that is, 11 *cis*-retinal—is bound to a lysine residue designated by "K" at position 296 in the seventh transmembrane segment. Each rhodopsin molecule is folded in three dimensions (Fig. 10, bottom), with the first and seventh transmembrane segments in close proximity. A normal folded molecule with seven transmembrane segments forms a pocket to hold a molecule of vitamin A, as represented in this cut-out view of the pocket. In broad terms, loops of the molecule near the amino terminus are involved in the folding of rhodopsin to form this pocket, whereas loops near the carboxy terminus bind to transducin as an early step in phototransduction.[89–91]

The first gene abnormality we discovered was a single-base substitution in codon 23 of the rhodopsin gene.[92] Representative sequencing gels are shown for a normal subject and five patients with autosomal dominant retinitis pigmentosa (Fig. 11). The lanes are labeled C, T, A, and G, corresponding to cytosine, thymine, adenine, and guanine. On the right are letters designating the three nucleotide bases making up each codon, the amino acid specified by that codon, and the number of that codon in the rhodopsin gene. Codon 23 normally reads CCC. Patients AD160, -133, -87, and -126 all show a single base change, or point mutation, seen as a band marked in brackets in the adenine lane, so that codon 23 reads CAC. A band in this position is not seen in the normal subject nor in patient AD12, neither of whom had this mutation. This mutation in codon 23 in one allele from CCC to CAC corresponds to a change from proline to histidine in the

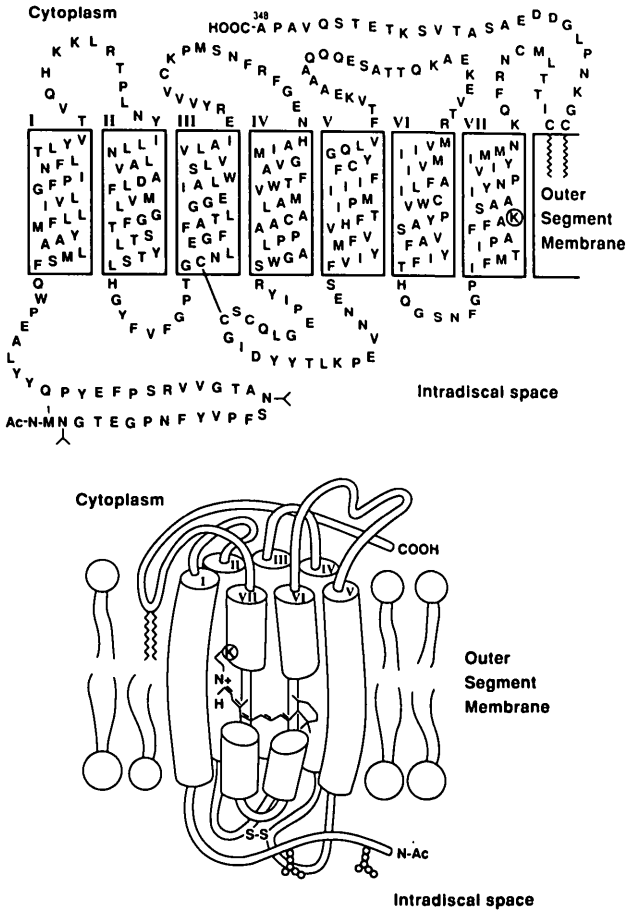


FIGURE 10. (Top) Model of the normal rhodopsin protein in a rod outer segment disk membrane. Each letter signifies an amino acid residue, using the standard single-letter code. A, alanine. R, arginine. N, asparagine. D, aspartic acid. C, cystine. E, glutamic acid. Q, glutamine. G, glycine. H, histidine. I, isoleucine. L, leucine. K, lysine. M, methionine. F, phenylalanine. P, proline. S, serine. T, threonine. W, tryptophan; Y, tyrosine; V, valine. (Bottom) Representation of a normal rhodopsin molecule folded in three dimensions to form a pocket to hold the vitamin A-derived chromophore (11-cis-retinal), which is covalently attached to a lysine residue, designated by "K" in the seventh transmembrane segment. (Modified from reference 96.)

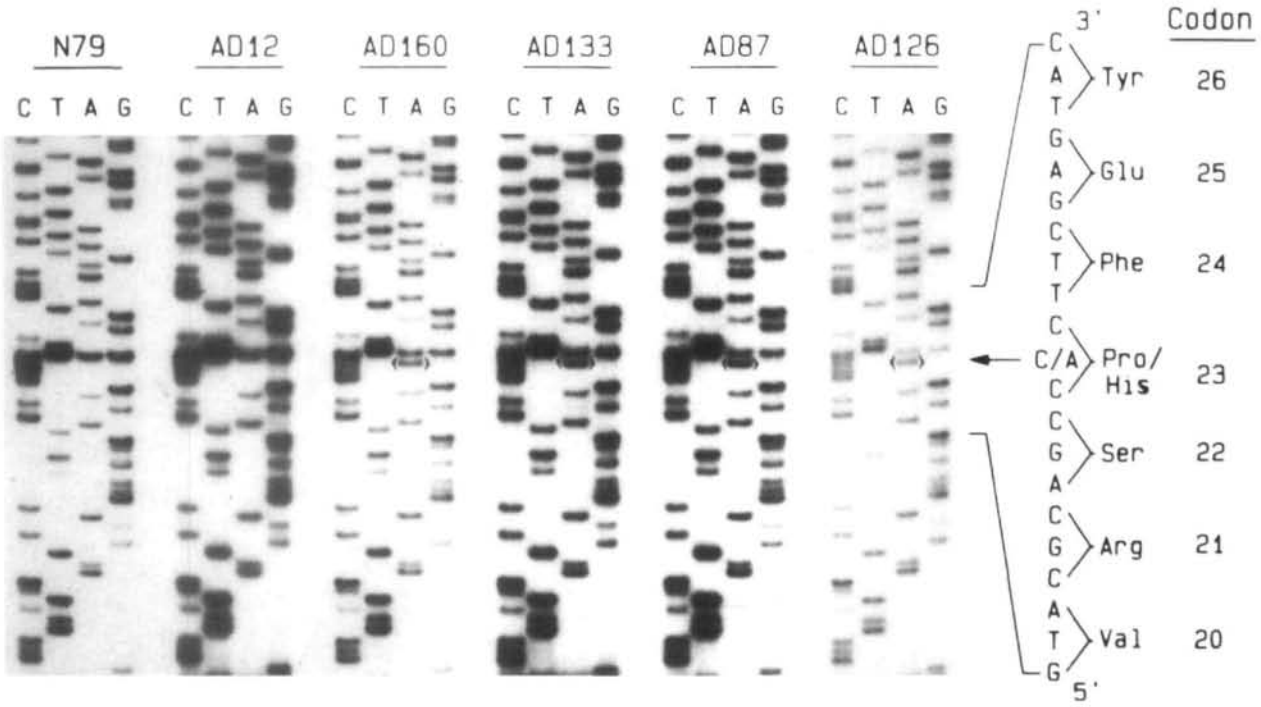


FIGURE 11. Nucleotide sequence of codons 20 to 26 of the human rhodopsin gene derived from leukocyte DNA of a normal individual (N79) and of five representative patients with dominant retinitis pigmentosa, identified by their molecular genetic numbers AD12, AD160, AD133, AD87, and AD126. The normal subject and patient AD12 show the normal sequence, whereas the other four patients are heterozygous for the cytosine-to-adenine transversion within codon 23 (CCC to CAC). In these four patients with this mutation, the single base change can be seen as a band marked in brackets. (From reference 95.)

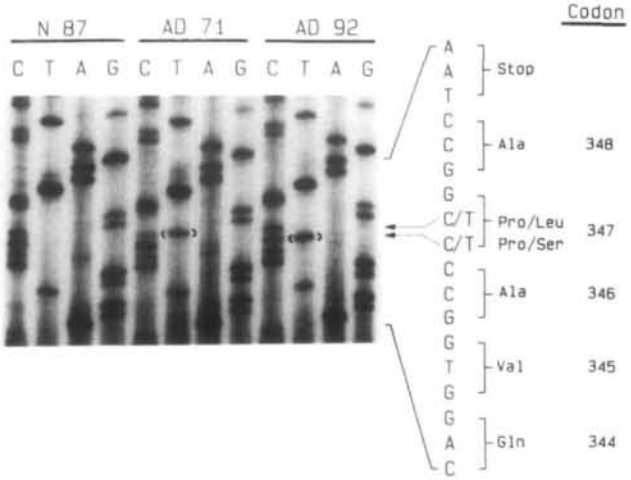


FIGURE 12. Heterozygous mutations of the rhodopsin gene in patients with autosomal dominant retinitis pigmentosa. Each set of four lanes represents DNA from one person. Brackets around two selected bands highlight the nucleotide bases present in patients AD71 and AD92 that are absent in the normal control. The mutation in patient AD71 changes codon 347 from CCG to CTG, corresponding to a change in the 347th amino acid from proline to leucine. The mutation in patient AD92 changes the same codon from CCG to TCG, corresponding to a change from proline to serine. (From reference 93.)

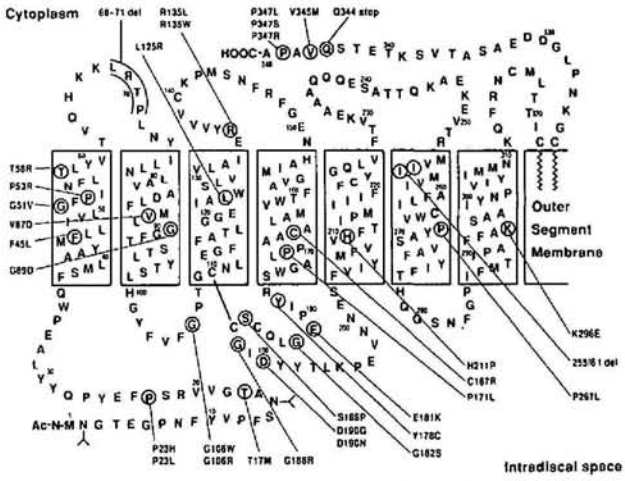


FIGURE 13. Schematic representation of the rhodopsin molecule. Amino acids known (as of May 1992) to be affected by mutations in families with autosomal dominant retinitis pigmentosa are circled or enclosed in brackets. Mutations at these sites are designated by the standard single-letter code —eg, P23H corresponds to Proline-23-Histidine. Single letter code, see Fig. 10 legend.

TABLE 2. Mutations Found in the Rhodopsin Gene in Patients with Autosomal Dominant Retinitis Pigmentosa*

No.	*Mutation*		*Normal Sequence†*	*References*
1.	Thr17Met	C → T	GCG ACG GGT	(99, 106, 109, 112–114)
2.	Pro23His	C → A	AGC CCC TTC	(92–95, 99, 104, 109–113)
3.	Pro23Leu	C → T	AGC CCC TTC	(99)
4.	Phe45Leu	T → C	ATG TTT CTG	(112, 113)
5.	Gly51Val	G → T	CTG GGC TTC	(99)
6.	Pro53Arg	C → G	TTC CCC ATC	(115)
7.	Thr58Arg	C → G	CTC ACG CTC	(93, 99, 101, 106, 108, 109, 112, 113)
8.	Del68-71	12 bp del	CTG CGC ACG CCT	(107)
9.	Val87Asp	T → A	ATG GTC CTA	(112, 113)
10.	Gly89Asp	G → A	CTA GGT GGC	(99, 112, 113)
11.	Gly106Trp	G → T	TTC GGG CCC	(112, 113)
12.	Gly106Arg	G → A	TTC GGG CCC	(116)
13.	Leu125Arg	T → G	GCC CTG TGG	(99)
14.	Arg135Leu	GG → TT	GAG CGG TAC	(106, 112, 113)
15.	Arg135Trp	C → T	GAG CGG TAC	(106, 112, 113)
16.	Cys167Arg	T → C	CGG TCG GCC	(99)
17.	Pro171Leu	C → T	CCC CCA CTC	(99)
18.	Tyr178Cys	A → G	AGG TAC ATC	(100, 112)
19.	Glu181Lys	G → A	CCC GAG GGC	(99)
20.	Gly182Ser	G → A	GAG GGC CTG	(109, 114)
21.	Ser186Pro	T → C	TGC TCG TGT	(99)
22.	Gly188Arg	G → A	TGT GGA ATC	(99)
23.	Asp190Asn	G → A	ATC GAC TAC	(99, 107)
24.	Asp190Gly	A → G	ATC GAC TAC	(99, 112, 113)
25.	His211Pro	A → C	GTC CAC TTC	(107)
26.	Ile255Del	3 bp del	GTC ATC ATC ATG	(98, 105)
27.	Pro267Leu	C → T	GTG CCC TAC	(109)
28.	Lys296Glu	A → G	GCC AAG AGC	(107)
29.	Gln344Stop	C → T	AGC CAG GTG	(106, 112, 113)
30.	Val345Met	G → A	CAG GTG GCC	(97, 99)
31.	Pro347Arg	C → G	GCC CCG GCC	(103)
32.	Pro347Leu	C → T	GCC CCG GCC	(93, 96, 98, 99, 102, 112, 113)
33.	Pro347Ser	C → T	GCC CCG GCC	(93, 99)

* Mutations published through May 1992.
† Affected bases are underlined.

23rd amino acid of the rhodopsin protein. We designate this mutation as rhodopsin, Proline-23-Histidine or Pro23His.

Figure 12 shows sequencing gels from a normal control and two patients who have point mutations at the other end of the rhodopsin gene in codon 347. The mutations are again seen as bands marked in brackets. Patient AD71 has a change in the second nucleotide and patient AD92 in the first, corresponding respectively to a change from proline to leucine or proline to serine in the 347th amino acid of the rhodopsin protein.[93]

The first four mutations we discovered in the rhodopsin gene in 28 of 150 patients from separate families were Pro23His, Pro347Leu, Pro347Ser, and Thr58Arg.[92–95] Frequencies by family were 12%, 5%, 0.5%, and 0.5%, respectively. Only one mutation was found in a given individual. Each mutation segregated perfectly with the disease, and we did not find these mutations in over 100 normal individuals who served as controls. Our group and others have now observed more than 30 mutations or small deletions in the rhodopsin gene (Table 2).[92–117] Amino acids affected by these mutations are encircled or bracketed on a schematic representation of the rhodopsin molecule (Fig. 13). The intradiscal, intramembranous, or intracytoplasmic domain of the molecule can be affected. These changes might affect the normal folding of the molecule, its capacity to bind vitamin A, the processes by which this protein is transported or incorporated into rod outer segments or some combination.[118] We estimate that 25 to 30% of patients with dominant retinitis pigmentosa in the United States have one of these mutations.

Clinical Findings in Patients With Rhodopsin Gene Mutations

Figure 14 shows full-field ERGs from a normal individual and from five patients from a family with

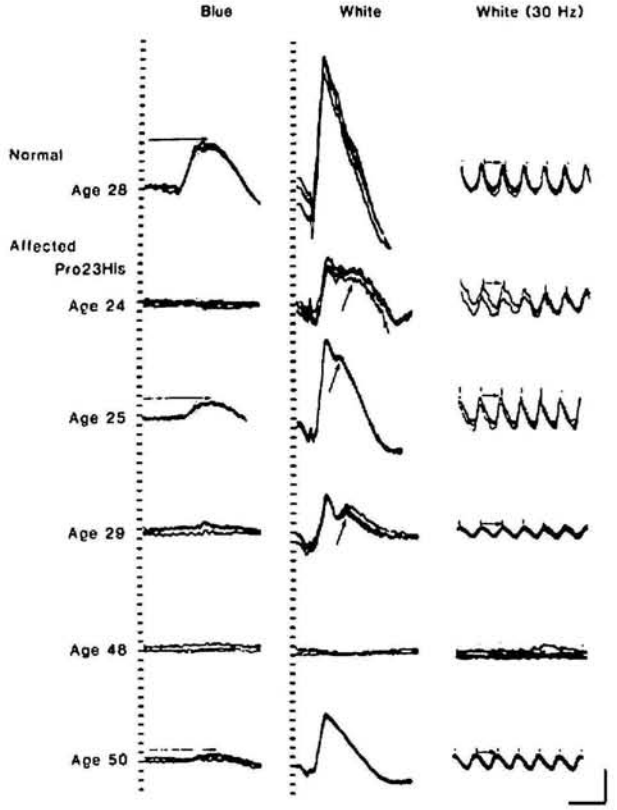


FIGURE 14. Full-field ERGs from an unaffected patient and five affected relatives in family 5850 with dominant retinitis pigmentosa and rhodopsin, Pro23His. Horizontal arrows designate implicit times. Oblique arrows designate delayed rod-dominated peaks. Calibration symbol (lower right) designates 50 ms horizontally and 100 μV vertically. (From reference 95.)

the Pro23His mutation.[95] Although all patients have reduced ERGs, considerable variability exists in the size of the responses at a given age. For example, an asymptomatic 50-yr-old patient has much larger responses than her night-deficient 48-yr-old sister. Fundus photographs from patients with the Pro23His mutation show either the typical intraretinal pigment around the periphery or little or no pigment (Fig. 15). A 33-yr-old patient from one family shows more pigment than patients ages 45 and 48 from other families. These ERGs and fundus photographs help substantiate that intrafamilial and interfamilial clinical variability exists at a given age among patients with this mutation.[95] Intra- and inter-familial variability also has been observed among patients with the Pro347Leu mutation.[96]

We have observed that patients with rhodopsin, Pro23His have, on average, 10-fold larger 0.5 Hz ERGs at their initial visits than those with rhodopsin, Pro347Leu, even though the former were, on average, 5 yr older than the latter (Table 3).[96] These differences

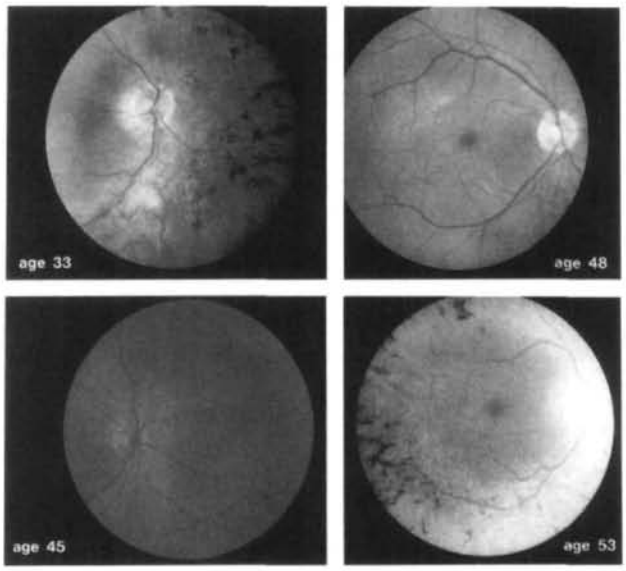


FIGURE 15. Representative fundus photographs from four patients with autosomal dominant retinitis pigmentosa with a C to A transversion in codon 23 of the rhodopsin gene to show variability with respect to the extent of intraretinal bone spicule pigmentation among patients with the same gene defect. (From reference 94.)

were statistically significant when each group was compared with a group of 120 patients that had neither mutation. Based on their mean amplitudes at the initial visit and assuming a loss of 16% of remaining ERG amplitudes per year based on our natural history study,[60] we have estimated that patients with the Pro23His mutation will be legally blind on average by age 70 whereas those with the Pro347Leu mutation will be legally blind on average by age 53.[96]

Transgenic Model of Human RP

Studies of transgenic mice have provided additional evidence that a rhodopsin mutation can cause photoreceptor degeneration. Mutant gene constructs derived from the DNA of affected patients have been injected into the fertilized eggs of mice to produce an animal model of the human disease. Light micrographs (Fig.

TABLE 3. Group Comparisons of Patients With Dominant RP

Parameter	*Group I Pro23His (n = 17)*	*Group II Pro347Leu (n = 8)*	*Group III Neither (n = 120)*
Mean age	37	32	32
0.5 Hz ERG*	14.4	1.7	5.3
30 Hz ERG*	5.5	0.5	1.6

* ERGs expressed as geometric mean amplitudes.
* Lower norm: 0.5 Hz, 350 μV; 30 Hz, 50 μV.
Group I vs Group III 0.5 Hz, $P < 0.01$; 30 Hz $P < 0.01$.
Group II vs Group III 0.5 Hz, $P < 0.01$; 30 Hz $P < 0.04$.

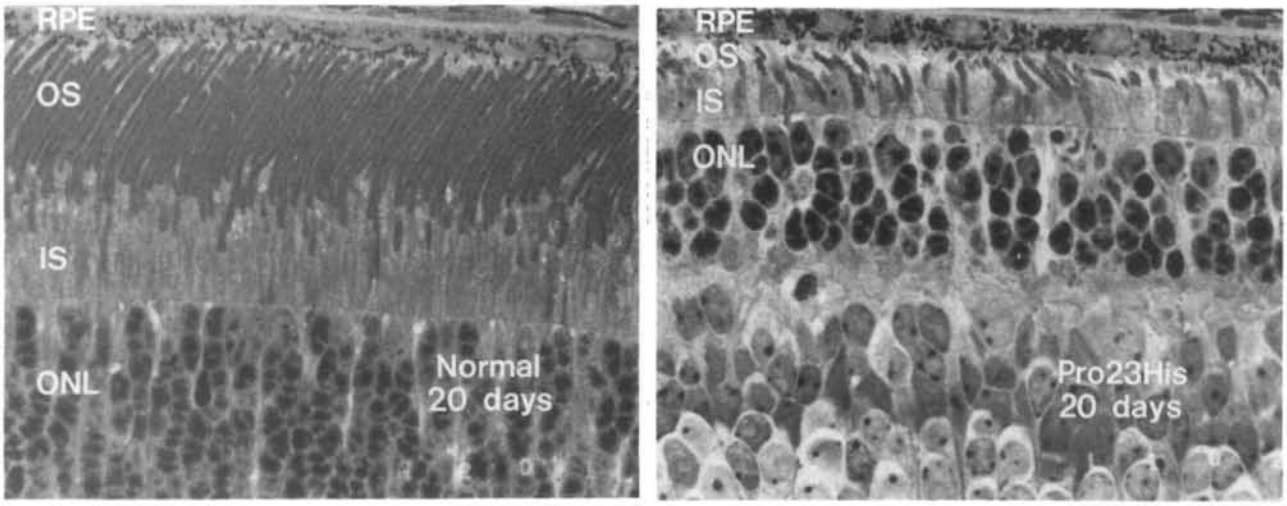
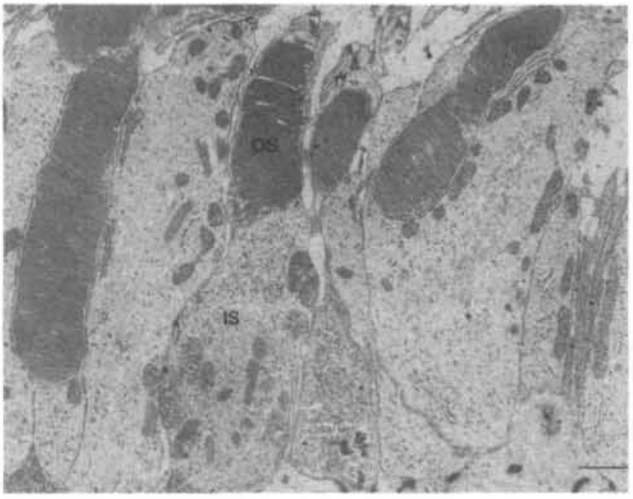
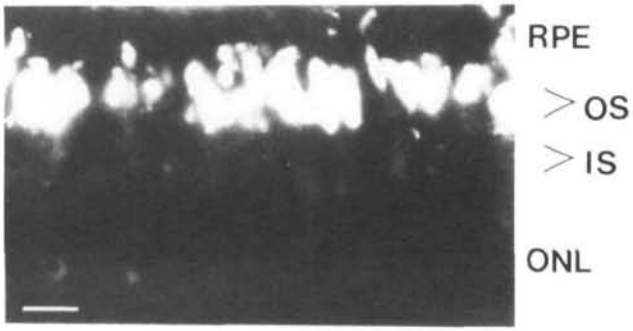


FIGURE 16. Light micrographs of retinas from a normal 20-day-old mouse (left) and from a transgenic mouse at the same age carrying the rhodopsin, Pro23His human transgene (right). The transgenic mouse shows a reduced outer nuclear layer (ONL) and shortened inner segments (IS) and outer segments (OS). Both show intact retinal pigment epithelium (RPE). (From reference 119.)





16) show that at postnatal day 20, a Pro23His transgenic mouse has about half the number of photoreceptor nuclei of an age-matched normal littermate that does not have the human rhodopsin transgene.[119] ERGs from these transgenic mice are profoundly reduced in amplitude and delayed with respect to implicit time. By electron microscopy, these mice show shortened outer segments with preserved outer segment discs and swollen inner segments (Fig. 17, top). Immunocytochemical analysis with a monoclonal antibody specific for human opsin shows the presence of human opsin in remaining outer segments of these murine photoreceptors at postnatal day 15 (Fig. 17, bottom). These results support the idea that a human rhodopsin gene construct with the Pro23His mutation leads to production of a human opsin that has a deleterious effect on photoreceptor function and viability.

FIGURE 17. (Top) Electron micrograph of retina from a Pro23His transgenic mouse (L line) at postnatal day 20 shows shortened outer segments (OS) and swollen inner segments (IS). (Bar = 1 μm). (Bottom) Human rod opsin expression within Pro23His-L line transgenic mouse retina at postnatal day 15. Unfixed cryostat sections of P23H-L retinas were stained with a monoclonal antibody (designated as rho 3A6) specific for human opsin. Staining can be seen in the developing outer segments (OS). (Bar = 15 μm). (From reference 119.)

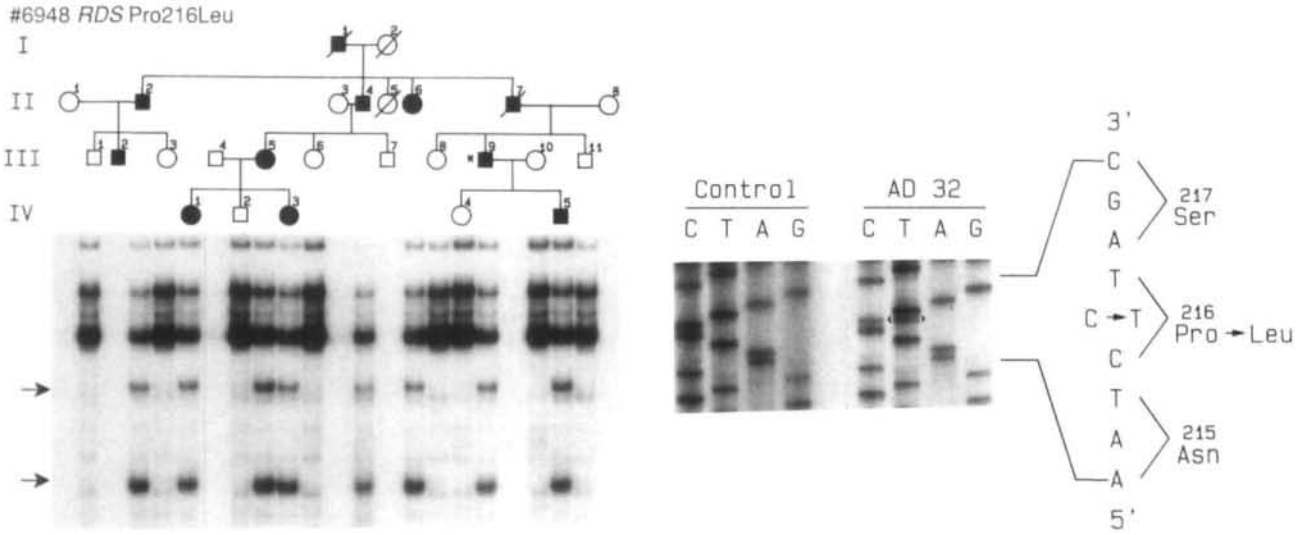


FIGURE 18. (Left) Pedigree of family 6948 with dominant retinitis pigmentosa and a mutation in the retinal degeneration slow (RDS) gene. Affected members are designated by filled symbols. Slashed symbols indicate deceased members. Analysis of leukocyte DNA with SSCP indicates cosegregation of variant bands (designated by arrows) with affected members. (Right) Sequencing gel from affected patient AD32 from family 6948 shows a C to T transition in the second base of codon 216, changing the specificity of this codon from proline to leucine. (From reference 15.)

RDS/Peripherin Gene Mutations in Dominant RP

Our group, as well as Humphries and colleagues in Ireland, also have reported mutations in the human homologue of the retinal degeneration slow (or RDS) gene, known to be abnormal in a strain of mice with a slow photoreceptor degeneration.[120-123] So far, we have reported three mutations of the RDS gene that cosegregate with dominant retinitis pigmentosa in separate families. Single strand conformation polymorphism (SSCP) analyses show mutant bands designated by arrows that cosegregate with the affected individuals in a family with RDS, Proline-216-Leucine (Fig. 18, left). A sequencing gel from a patient shows a heterozygous C to T transition in codon 216 that corresponds to a change from proline to leucine in the 216th amino acid of the RDS protein, also called peripherin (Fig. 18, right).

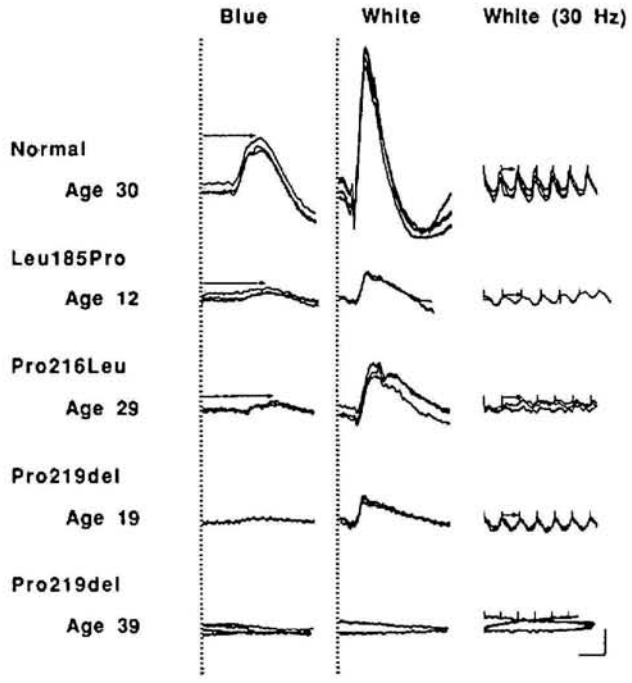


FIGURE 19. Full-field ERGs from a normal subject and four patients with dominant retinitis pigmentosa and RDS mutations. Calibration symbol (lower right) designates 50 msec horizontally and 100 μV vertically. (From reference 15.)

ERGs from patients with RDS mutations have shown reductions of rod and cone amplitudes with delays in implicit times (Fig. 19). The RDS protein is composed of 346 amino acid residues. Its function appears to be maintenance of rod and cone outer segment disc structure. Amino acids affected by mutations that we have reported so far (eg, Leu185Pro, Pro216Leu, and Pro219del) are encircled and further identified with arrows on a schematic representation of this protein (Fig. 20).[124] Substitutions between proline and leucine or the loss of a proline residue are nonconservative changes that would be expected to have major effects on the structure of this protein. Each RDS mutation has segregated perfectly with the disease. We have not seen these mutations in normal individuals or in patients with rhodopsin mutations. Therefore, we conclude that some cases of dominant retinitis pigmentosa are the result of mutations in the RDS/peripherin gene on chromosome 6.

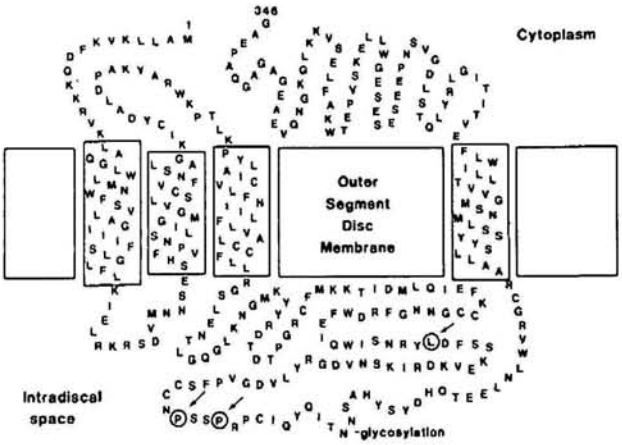


FIGURE 20. Schematic model of the peripherin protein. The molecule is thought to traverse the rod outer segment membrane four times. Amino acids corresponding to RDS mutations so far identified in some families with dominant retinitis pigmentosa are circled. (Adapted from Connell GJ, Bascom R, Molday L, Reid E, McInnes RR, and Molday RS: Photoreceptor peripherin is the normal product of the gene responsible for retinal degeneration in the rds mouse. *Proc Natl Acad Sci USA* 1991;88:723.)

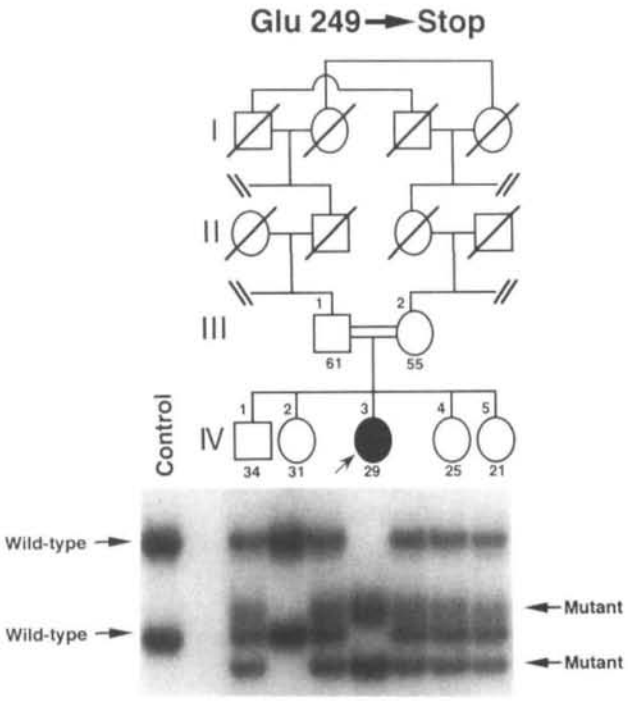


FIGURE 21. Cosegregation of autosomal recessive retinitis pigmentosa with variant bands detected by SSCP analysis. The SSCP analysis of each living family member is beneath their symbol in the schematic pedigree. The arrows on the left of the autoradiograph designate the position of the single-strand fragments with wild-type sequence as shown for the normal control in the left lane. The arrows on the right depict the fragments with the G to T transversion. The affected member of the pedigree, patient AR106, is designated by the filled symbol and arrow. (From reference 125.)

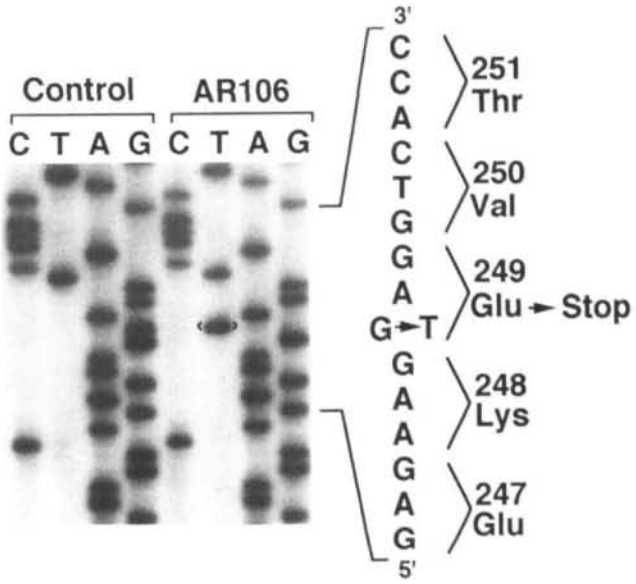


FIGURE 22. Nucleotide sequence of variant bands detected by SSCP analysis. Patient AR106 has a homozygous G to T transversion in the first base of codon 249 that results in a nonsense mutation. The 5′ end of the sense sequence is at the bottom of the figure and the 3′ end is at the top. (From reference 125.)

Rhodopsin Gene Defect in Autosomal Recessive RP

We recently identified a rhodopsin gene defect in a family with autosomal recessive retinitis pigmentosa;[125] the pedigree is illustrated in Figure 21. Arrows on the left designate positions of single strand fragments for a normal control with the wild type sequence. Arrows on the right of this gel indicate the positions of mutant fragments. The affected 29-yr-old woman (designated by the solid symbol) shows two mutant bands and no wild-type bands. In contrast, both parents display four bands—that is, two mutant and two wild type bands and therefore are heterozygous for this mutation. Three of four siblings also show four bands and therefore are heterozygotes. Direct genomic sequencing of the amplified DNA from the fully affected female shows a homozygous G to T transversion in codon 249 that would result in a stop codon (Fig. 22). This mutation, designated as rhodopsin, Glutamic acid-249-Stop, would be expected to encode a markedly truncated opsin protein that would be functionally inactive because of the absence of the sixth and seventh transmembrane domains, including the vitamin A attachment site. This defect was found in 1 of 126 separate families with recessive retinitis pigmentosa. It was not found in normal controls.[125]

The complete loss of functional rhodopsin would be consistent with the early onset of night blindness. Indeed, the affected woman, who carries this mutation

homozygously, reported night blindness as long as she could remember and showed constricted fields and very reduced ERGs at her initial examination by us at age 29 (Fig. 23). Her normal sibling (designated as wild type) had normal ERGs. Heterozygous parents and siblings, represented in the bottom two rows, had normal fundi but had rod amplitudes to dim blue light near the lower limit of normal, raising the possibility of a defect in rod function. Rod ERG intensity-amplitude functions revealed that the carriers in this family required, on average, 0.5 log unit more light to reach the half-maximal rod b-wave amplitude than controls, but had the same amplitudes to the maximal light stimulus as the controls (Fig. 24). Similar abnormal rod ERG intensity-amplitude functions have been reported by Johnson and Pak in *Drosophila* rhodopsin mutants that have diminished amounts of rhodopsin in their photoreceptors,[126] leading to the proposal that human carriers of Glu249Stop also have a diminished amount of rhodopsin in their photoreceptors.

Summary and Future Directions

In summary, retinitis pigmentosa can be detected early in life based on reduced full-field ERGs with delayed b-wave implicit times. Relatives of affected patients with normal ERGs would not be expected to develop retinitis pigmentosa later. The course of the disease can be monitored with computer-averaged and narrow-bandpassed filtered responses. Patients, ages 6 to 49, lose on average 16% of remaining full-field ERG amplitude per year. Cones and rods appear to be functioning normally for their numbers and their amounts of remaining visual pigment.

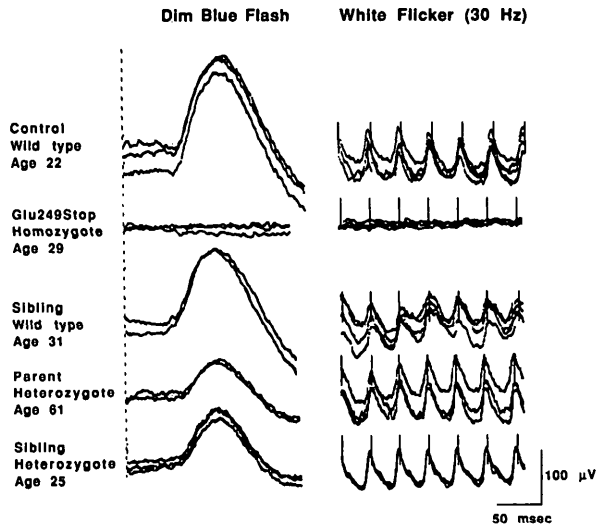


FIGURE 23. Full-field ERGs from an unaffected control; patient AR106 (IV-3), who is homozygous for Glu249Stop; a female sibling (IV-2) with wild-type (ie, normal) rhodopsin alleles; the father (III-1), who is heterozygous for Glu249Stop; and a female sibling (IV-4) who is heterozygous for Glu249Stop. Under these test conditions, normal amplitudes are ≥100 μV (left column) and ≥50 μV (right column). (Adapted from reference 125.)

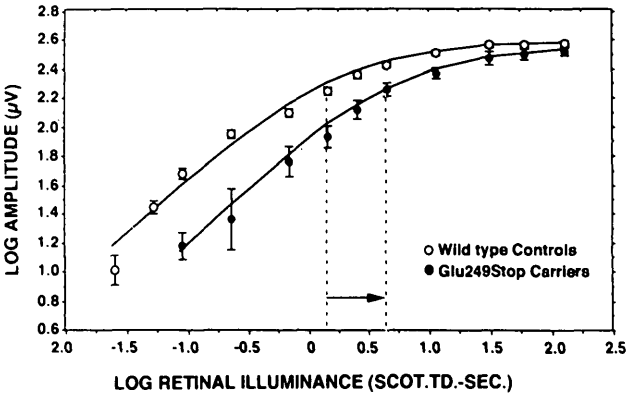


FIGURE 24. Mean log rod b-wave amplitude (±SEM) versus log retinal illuminance plotted for 17 normal control individuals with wild-type rhodopsin alleles (open circles) and five heterozygous carriers of the rhodopsin *null* mutation (filled circles). Vertical dashed lines designate the log retinal illuminances required for half-maximal responses, and the horizontal arrow designates the log retinal illuminance increase for the carriers. SCOT TD-SEC, scotopic troland-seconds. (From reference 125.)

Rhodopsin or RDS gene mutations exist in some forms of retinitis pigmentosa. Each mutation has segregated perfectly with the disease, as defined by an abnormal ERG. These data, as well as the findings from animal models, support the idea that mutations in these genes cause some forms of retinitis pigmentosa. Patients with rhodopsin, Proline-347-Leucine appear to have a more severe disease than patients with rhodopsin, Proline-23-Histidine. Variability of clinical expression exists at a given age among patients with these mutations, suggesting that some factors other than the gene defect itself may be affecting the course.

One of the challenges we face is to find the gene abnormalities in all forms of retinitis pigmentosa. The creation of transgenic models and cell culture systems[113,127,128] with these gene defects should help define pathogenetic mechanisms and aid the search for treatments. Clinically, we must define the phenotypes of patients with known genotypes and determine the natural course of these subtypes. Risk factor analyses of well-defined populations studied over time may reveal ameliorating or aggravating factors associated with the course of the disease, with possible implications for therapies.

Much work remains to be done. It still is my belief, as it was 20 years ago, that coordinated programs of clinical and basic research offer the greatest hope of finding treatments for patients with this group of diseases.

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