

Retinal Degenerations: Planning for the Future

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1 Introduction

Retinal degenerations are a leading cause of blindness in many parts of the world (Bunker et al., 1984; Grondahl, 1987; Berson, 1993; Klein et al., 1995; Attebo et al., 1996; Klaver et al., 1998; Novak-Laus et al., 2002). In the United States an estimated 6 million people have age-related macular degeneration with a decrease in central vision after age 60. About 100,000 people have retinitis pigmentosa or a related disease with loss of side and night vision in adolescence; they often develop tunnel vision by age 40 and lose central vision by age 60. Some 20% of patients with retinitis pigmentosa have associated hearing loss and this combination is called Usher syndrome. With increased life expectancy, the problem posed by these conditions is magnified, as affected individuals will have to endure more years of visual loss unless new treatments are found. This plenary lecture provides an overview of clinical findings and molecular genetic abnormalities in these diseases and reviews current treatments for these conditions. Selected studies of animal models of retinal degenerations will be summarized. Some proposed future therapies for human retinal degenerations will also be considered.

2 Human Retinal Degenerations: Some Clinical Findings

Fundus photographs are illustrated from patients with some forms of retinal degeneration (Fig. 1). Patients with the dry form of age-related macular degeneration have white deposits called drusen and eventual atrophy. Patients with the wet or leaky form of age-related macular degeneration show a disturbance in the appearance of the macula with hemorrhages, exudates, and scarring. Most patients with age-related macular degeneration have the dry form, but about 8% of those with the dry form

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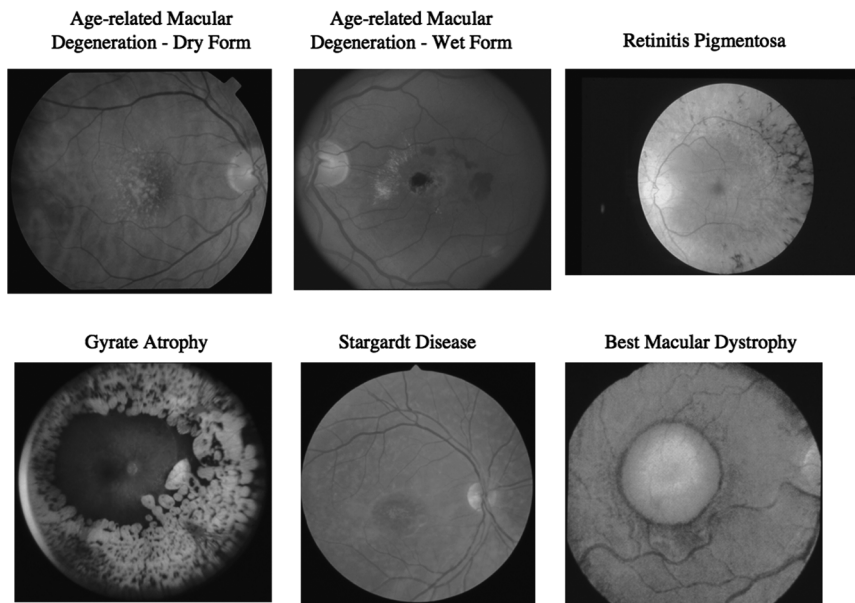


Fig. 1 Fundus photographs from patients with retinal degenerations

develop the more devastating wet form each year. Patients with retinitis pigmentosa develop pigment in a bone spicule configuration around the midperipheral retina for which the condition was named. Gyrate atrophy of the choroid and retina, a related night-blinding disorder, is characterized by areas of atrophy around the periphery that proceed toward the macula with eventual blindness often by age 50. Macular degeneration can also affect children. For example, one form called Stargardt disease results in a disturbance of macular pigmentation that can lead to visual decline by age 10. Another form called Best macular dystrophy shows a central egg-yolk like deposit that can rupture in some cases with a dramatic decline in central vision. Although these diseases appear clinically distinct on fundus examination, all affect cone and rod photoreceptors as well as the underlying retinal pigment epithelium; therefore, research on any one of them could enhance the understanding of the cellular mechanisms involved in the others.

Most retinal degenerations affect both cones and rods. To understand these conditions, it is important to know how the cones and rods are distributed across the retina and their role in vision. The cones are normally in highest concentration in the central retina or macula but cones are present across the peripheral retina as well (Fig. 2) (Osterberg, 1935). More than 90% of the cones are outside the central 10 degrees. Cones allow us sharp visual acuity and full side vision in color. Rods are also present across the retina except in the central macula and the highest concentration of rods is located 20–40 degrees eccentric to the foveola. Rods allow us to see large letters and provide us with a full visual field in shades of gray. Whereas the cones allow us to see under daylight conditions, the rods permit us to see under moonlight or starlight

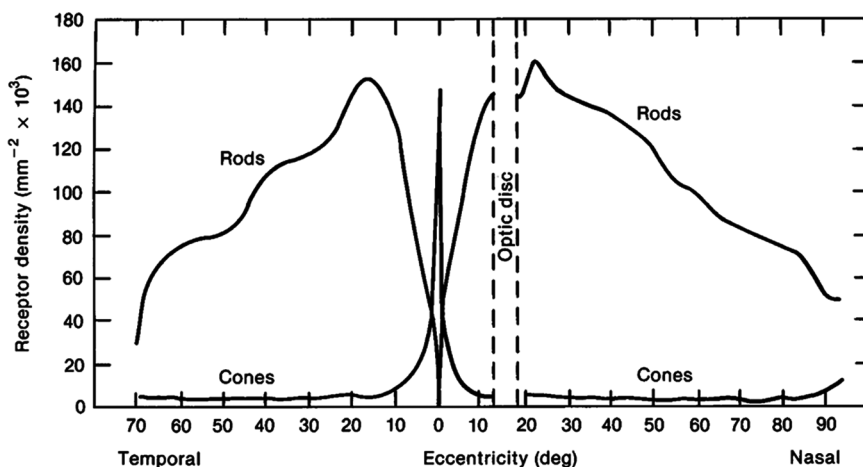


Fig. 2 Distribution of rods and cones in the normal human retina. Corresponding perimetric angles from the fovea at 0° are given. (After Osterberg from Pirenne MH: *Vision and the eye*, London. Chapman and Hall, 1967)

conditions. The normal human retina has over 100 million rods and 7 million cones. In the case of macular degeneration, both the macular cones and macular rods are compromised. In the case of retinitis pigmentosa or gyrate atrophy, both rods and cones across all or nearly all the retina are affected in the early stages as monitored by full-field electroretinograms (ERGs) (Berson, 1993).

Some hereditary retinal diseases selectively affect cone or rod function across the entire retina. For example, patients with congenital achromatopsia, who are born without color vision, have reduced cone function but retain normal rod function for their entire lives. Patients with congenital stationary night blindness are born with rod malfunction but retain normal or nearly normal cone function for their entire lives. It is important to note that these patients with stationary night blindness do not develop rod degeneration. In contrast, patients with retinitis pigmentosa develop rod degeneration and eventually cone degeneration as well. These findings lead to the conclusion that rods can live without cones but cones cannot live without rods. Why do we have a rod-dominant retina when we function most of the time with our cones? The answer has relevance to treating some retinal degenerations and we will return to this question later.

3 Molecular Genetic Abnormalities

Over 100 genes have been implicated in human hereditary retinal degenerations. For the subset of diseases called retinitis pigmentosa, over 45 causative genes have been identified that account for 50–60% of all cases. These genes can be sub-classified based on the known or presumed function of encoded proteins. Genes may affect the phototransduction cascade, vitamin A metabolism, photoreceptor structure, signaling or cell-cell interactions, RNA intron splicing factors, intracellular transport

of proteins, maintenance of cilia or ciliated cells with a possible role in intracellular trafficking, regulation of the carbon dioxide-bicarbonate balance, phagocytosis, and other yet to be defined functions of the photoreceptors and pigment epithelium (Hartong et al., 2006). The most common genes causing retinitis pigmentosa are the rhodopsin (*RHO*) gene, the *USH2A* gene, and the retinitis pigmentosa GTPase regulator (*RPGR*) gene, each of which accounts for about 10% of all cases in North America (Hartong et al., 2006). In patients with gyrate atrophy, mutations have been discovered in the ornithine amino transferase (*OAT*) gene that result in a defect in the metabolism of ornithine (Ramesh et al., 1991). A current listing of mutations that cause retinitis pigmentosa and allied hereditary retinal diseases is maintained on the world wide web at www.sph.uth.tmc.edu/RetNet/.

With respect to age-related macular degeneration, general agreement exists that this condition is seen more frequently among individuals who have affected relatives, but a causative gene (or genes) has yet to be discovered. A DNA change in a gene encoding complement factor H increases the risk of developing age-related macular degeneration by almost four-fold (Edwards et al., 2005; Haines et al., 2005; Klein et al., 2005). In the case of juvenile macular degeneration, early detection by DNA analysis is now possible for Stargardt disease based on mutations in the *ABCA4* gene (Allikmets et al., 1997; Briggs et al., 2001) and for Best macular dystrophy based on mutations in the Bestrophin gene (Marquardt et al., 1998); although early diagnosis is possible, no treatment is yet known for these conditions.

4 Treatment of Retinal Degenerations

Progress has been made in treating some retinal degenerations. Briefly stated, the dry form of age-related macular degeneration can be slowed with a combination of beta-carotene, vitamin C, vitamin E, zinc, and copper; patients on this regimen have a 25% reduction in the risk of developing advanced macular degeneration (Age-Related Eye Disease Study Research Group, 2001). The wet form can be stabilized by laser therapy (Macular Photocoagulation Study Group, 1993). Intravitreal injections of steroids, Macugen[®], Lucentis[®], or more recently Avastin have been shown to interfere with blood vessel growth and can either arrest loss of vision or in some cases improve vision (Rosenfeld, 2006; Rosenfeld et al., 2006). Vitamin A palmitate can slow the common forms of retinitis pigmentosa (Berson et al., 1993). Gyrate atrophy, associated with an elevated serum ornithine level, is treatable with a low-protein, low-arginine diet or vitamin B₆ that lower the serum ornithine level toward normal with slowing of progression of the atrophy (Kaiser-Kupfer et al., 1991).

Some rare forms of retinitis pigmentosa have yielded to treatment when initiated at an early stage. Specifically, vitamins A and E have been used successfully to restore and maintain retinal function in hereditary abetalipoproteinemia (i.e. Bassen-Kornzweig disease) in the short term (Gouras et al., 1971; Bishara et al., 1982). Over the long term, some progression has been observed in these patients despite use of vitamin A and vitamin E (Chowers et al., 2001). A low phytol-low phytanic acid diet has resulted in stabilization of retinal function in patients with phytanic acid oxidase

deficiency (i.e. Refsum disease) (Refsum, 1981; Hungerbuhler et al., 1985). Vitamin E supplementation has been used to stabilize retinal function in patients with isolated familial vitamin E deficiency (Yokota et al., 1997). In these conditions knowledge of the biochemical abnormalities has led to rational approaches to therapy.

5 Clinical Trials for Retinitis Pigmentosa

The typical forms of hereditary retinitis pigmentosa have been successfully treated with nutritional interventions. While studying the natural course of retinitis pigmentosa (Berson et al., 1985), those patients self-treating with a separate capsule of vitamin A and/or vitamin E showed a smaller decline in ERG amplitude than those taking a multi-vitamin or no vitamin supplement (Berson et al., 1993). The critical intake for a therapeutic effect appeared to be 16,500 IU/day of preformed vitamin A (diet plus supplements). Most patients taking a separate capsule of vitamin A were also taking a separate capsule of vitamin E so that it could not be determined whether one or the other or the combination was potentially therapeutic. No relationship was found between intake of other vitamins and ERG decline in these patients. Based on these preliminary findings, a randomized, controlled, double-masked trial was conducted for 601 patients, aged 18–49 years, from across the United States and Canada. Patients were randomized to 15,000 IU of vitamin A palmitate (Group A), 400 IU of vitamin E as *dl*-alpha-tocopherol (Group E), the two vitamins combined (Group A+E), or a control group receiving trace amounts of both (Group Trace). Patients were followed annually for 4–6 years. The main outcome variable was the full-field 30-Hz cone ERG (Fig. 3). Secondary outcome variables were visual field area with a V-4e white test light on the Goldmann perimeter and ETDRS visual

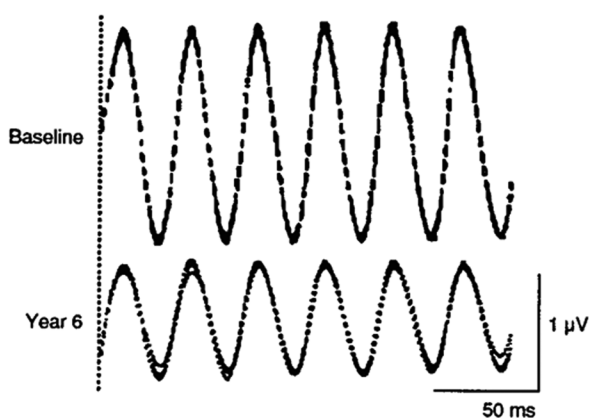


Fig. 3 Narrow bandpassed, computer-averaged full-field cone ERGs in response to 30Hz flashes ($n=256$ sweeps with 6 responses per sweep) from a representative patient with retinitis pigmentosa at baseline and 6 years after baseline. Three consecutive averages are superimposed. The broken vertical line indicates the onset of the train of flashes. Lower norm = 50 μ V, virtual blindness ≤ 0.05 μ V. (From Berson et al., Arch Ophthalmol 111:763, 1993)

acuity. Results were monitored by an independent Data and Safety Monitoring Committee selected by the National Eye Institute (Berson et al., 1993a).

Among all randomized patients those taking vitamin A, 15,000 IU/day, showed a slower rate of decline in remaining cone ERG amplitude than those not on this dose ($p=0.01$). The vitamin A treatment effect was more significant ($p<0.001$) in a subset of 354 patients with slightly higher initial cone amplitude ($\geq 0.68 \mu\text{V}$), designated as the higher amplitude cohort, who could be followed with greater precision. In this cohort a significant adverse effect ($p=0.04$) of vitamin E was also observed. Mean decline in 30 Hz ERG amplitude by vitamin A intake showed that the least decline occurred with an intake of about 18,000 IU/day (diet plus supplements) and that higher intake conferred no greater benefit (Fig. 4). No treatment effect could be detected with respect to visual acuity over the time interval of this study (Berson et al., 1993). In a subset of 125 patients who could perform visual fields with great precision, a significant beneficial effect of vitamin A was observed with visual field testing (Berson, 1998).

These results have led to the recommendation that most adults with retinitis pigmentosa should take vitamin A palmitate, 15,000 IU/day, and avoid high doses of vitamin E such as the 400 IU/day used in this trial (Berson et al., 1993a,b; Berson, 1998). Beta-carotene, the precursor of vitamin A, is not predictably converted to vitamin A and, therefore, is not a suitable substitute for vitamin A in the context of this treatment. No significant toxic effects have been observed with this treatment (Sibulesky et al., 1999). This treatment is not recommended for women who are pregnant or planning to become pregnant because of the increased risk of birth defects among patients on high doses of vitamin A. Since high dose vitamin A supplementation has been associated with a slight increase in the risk of hip fracture in post-menopausal women (Feskanich et al., 2002) and men over age 49

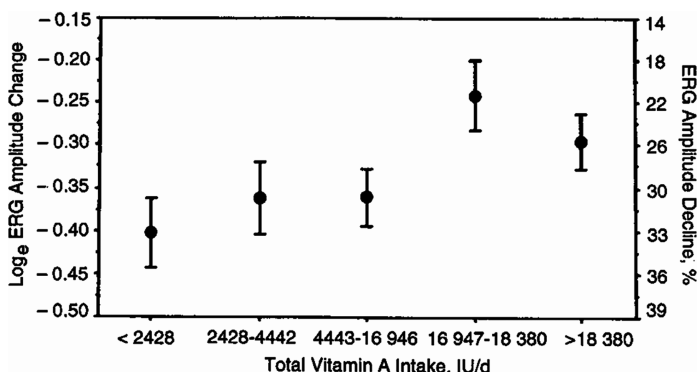


Fig. 4 Mean \pm SE decline from baseline in 30Hz ERG amplitude by total vitamin A intake (diet plus capsules) irrespective of randomization assignment for all patients in the higher-amplitude cohort. The mean decline was calculated as the mean of screening and baseline minus the mean of all follow-up visits by quintile of total vitamin A intake averaged over all visits. Sample sizes were $n=69$, $n=72$, $n=74$, $n=65$, and $n=74$ for the lowest to highest quintiles of total vitamin A intake. Vertical bars indicate SEs. (From Berson et al. Arch Ophthalmol, 111:769, 1993a)

years (Michaelsson et al., 2003), patients who take this dose of vitamin A should be advised to monitor their bone health.

While conducting the vitamin A trial, an inverse relationship was observed in a subset of these patients between red blood cell docosahexaenoic (RBC DHA) levels and rate of progression of retinitis pigmentosa; those with higher RBC DHA levels had a significantly slower rate of decline in ERG amplitudes than those with lower levels. This led to a second randomized, controlled, double-masked trial of DHA supplementation for retinitis pigmentosa. Two hundred and twenty-one patients, aged 18–55 years, were randomly assigned to either 1200 mg/day of DHA or control fatty acid capsules; all were given 15,000 IU/day of vitamin A as retinyl palmitate. The primary outcome measure was the total point score in the Humphrey Field Analyzer monitored annually with the 30-2 program and a size V white target. Cone ERG amplitudes, ETDRS visual acuities, and combined visual fields (30-2 plus 30/60-1 programs) were assessed annually as secondary outcome measures. This study allowed at least 4 years of follow-up for each patient. Results were monitored by an independent Data and Safety Monitoring Committee selected by the National Eye Institute (Berson et al., 2004).

The primary analyses of the total study population did not reveal, on average, a beneficial effect of DHA, 1200 mg/day, for patients with retinitis pigmentosa on vitamin A over a 4-year interval as monitored by visual field sensitivities, ERG amplitudes, and visual acuities. No toxic effects attributable to DHA or vitamin A were noted (Berson et al., 2004). Subgroup analyses, however, showed that for the patients with retinitis pigmentosa beginning vitamin A therapy for the first time at the beginning of this trial (i.e. 30% of the study population), addition of DHA, 1200 mg/day, slowed the course of disease for 2 years as monitored by visual field sensitivities and cone ERG amplitudes (Table 1). Furthermore, among patients in the control group on vitamin A for at least 2 years (but not on DHA capsules), a diet rich in omega-3 fatty acids (≥ 0.20 grams per day) of which DHA is a major constituent slowed the decline in visual field sensitivity by 40–50% per year (Table 2). In the entire study population red blood cell DHA levels were inversely related to rate of decline in total field sensitivity over 4 years (Berson et al., 2004a).

The lack of a beneficial effect of DHA, 1200 mg/day, on the course of retinal degeneration among adults with typical retinitis pigmentosa on vitamin A precludes any general recommendation of DHA capsules for such patients. However, results from subgroup analyses have led to the recommendation that adults with typical retinitis pigmentosa already taking vitamin A palmitate, 15,000 IU/day, should also eat 1–2 three-ounce servings per week of omega-3 rich fish (salmon, tuna, mackerel, herring, or sardines) of which DHA is a major constituent. About three months after starting this omega-3 rich fish diet, thereby allowing sufficient time for RBC turnover, patients should have a measurement of their fasting RBC DHA through their physician to confirm that the RBC DHA level is at least 4% of total RBC fatty acids, as such patients have, on average, a slower rate of decline of visual field sensitivity than those with lower levels. With respect to adults starting vitamin A for the first time, they should also take DHA capsules 600 mg twice each day for 2 years to shorten the interval for vitamin A to achieve its benefit. After two years they should continue vitamin A palmitate, 15,000 IU/day, stop the DHA capsules

Table 1 Annual rate of decline for measures of ocular function by treatment group and vitamin A status prior to entry over a 4-year interval

	On vitamin A prior to entry		
	DHA + A	Control + A	P†
HFA 30-2 field, dB/y	39.41 ± 3.76 (74)	30.26 ± 3.93 (68)	.09
HFA total field, dB/y‡	61.01 ± 5.17 (74)	48.13 ± 5.39 (68)	.08
30-Hz ERG, log _e	0.11 ± 0.01 (75)	0.10 ± 0.01 (67)	.34
% Decline per year §	10.57	9.23	
ETDRS visual acuity letters per year	0.67 ± 0.13 (75)	0.68 ± 0.14 (68)	.96
	Not on vitamin A prior to entry		
	DHA + A	Control + A	P†
HFA 30-2 field, dB/y	30.70 ± 6.48 (29)	52.50 ± 5.99 (34)	.01
HFA total field, dB/y‡	47.16 ± 10.56 (28)	82.49 ± 9.58 (34)	.01
30-Hz ERG, log _e	0.08 ± 0.02 (26)	0.14 ± 0.02 (33)	.03
% Decline per year §	8.05	12.99	
ETDRS visual acuity letters per year	0.82 ± 0.26 (29)	0.69 ± 0.24 (34)	.70

Unless otherwise indicated, data expressed are means ± SE; numbers of patients sampled are designated in parentheses. Patients received either 1200 mg/d of docosahexaenoic acid plus 15,000 IU/d of vitamin A (DHA + A) or control capsules plus 15,000 IU/d of vitamin A (control + A). †P value calculated from PROC MIXED (SAS Institute Inc, Cary, NC) analysis comparing rates of decline in both treatment groups. ‡Total field sensitivity consists of 30-2 and 30/60-1 total point scores combined when both are available. § Derived from $100 \times [1 - \exp(\text{mean log change})]$. Significant statistical interactions between the effect of DHA and whether or not patients were on vitamin A prior to entry were observed for HFA 30-2 field ($P=.002$), HFA total field ($P=.001$), and 30-Hz ERG ($P=.02$) (From Berson et al., Arch Ophthalmol 122:1308, 2004)

Table 2 Annual decline in visual field sensitivity in the control group as a function of dietary ω -3 fatty acid intake among patients on vitamin A prior to entry

Dietary ω -3 Intake	Years 0 to 4	Years 0 to 2	Years 2 to 4
30-2 Condition, dB			
<0.20 g/d	39.2 ± 5.5 (35)	32.9 ± 8.2 (35)	44.5 ± 7.5 (35)
≥ 0.20g/d	20.8 ± 5.7 (33)	14.3 ± 8.4 (33)	20.1 ± 7.7 (33)
P value ‡	.02	.12	.03
Total condition, dB §			
<0.20 g/d	57.8 ± 7.0 (35)	44.7 ± 12.4 (35)	72.9 ± 10.5 (35)
≥ 0.20g/d	37.9 ± 7.2 (33)	25.0 ± 12.7 (33)	39.3 ± 10.8 (33)
P value ‡	.05	.27	.03

Unless otherwise indicated, data expressed are means ± SE; numbers of patients sampled are designated in parentheses. Patients in the Control group received control capsules plus 15,000 IU/d of vitamin A. †Expressed as the mean of grams per day of all visits. ‡Based on PROC MIXED (SAS Institute Inc, Cary, NC) analysis comparing rates of decline in high versus low omega-3 fatty acid intake groups. § Total condition consists of the sum of 30-2 and 30/60-1 conditions when both are available. (From Berson et al., Arch Ophthalmol 122:1310, 2004b)

(because a slight tendency toward adversity over the long-term has been observed among patients concurrently on vitamin A), and also eat 1–2 three-ounce servings of omega-3 rich fish each week. They should then have a measurement of fasting RBC DHA through their physician three months later to be certain that the level is at least 4% of total RBC fatty acids. It has been estimated that the combination of vitamin A palmitate with an oily fish diet, on average, could provide almost 20 additional years of vision for the average patient who starts this regimen in their mid-thirties (Berson et al., 2004a).

It is remarkable that treatment with a single vitamin, namely vitamin A, has proven to be beneficial on average for patients with retinitis pigmentosa even though they are losing vision due to many different gene defects. To help explain this, we return to the question raised earlier, namely why do we have a rod-dominant retina when we function most of the time with our cones? Why are we using only 7% of our photoreceptors, namely our cones, to look at each other in this room; what are our rods doing under daylight conditions?

We know that both cones and rods are activated by light. The cone signal arrives first at the optic nerve and we see each other in color. The rod response to light is not wasted as the rods, in response to light, expel a twisted form of vitamin A, namely all-trans-retinol, that is reconfigured by nearby support cells called Müller cells. The reconfigured vitamin A, that is, 11-cis-retinol, is then transported to cones which contain a dehydrogenase; the cones have the capacity to further convert the vitamin A from 11-cis-retinol to 11-cis-retinal (Mata et al., 2002), the form needed for visual excitation. Therefore, one can hypothesize that under daylight conditions rods are giving cones vitamin A via Müller cells. Interphotoreceptor retinoid binding protein (IRBP) transports vitamin A between these cells and from the pigment epithelium to these cells as well. Release of vitamin A from IRBP requires DHA (Wolf, 1998), which is present in high concentration in an oily fish diet. Rod degeneration results in a retinal deficiency of vitamin A and DHA in retinitis pigmentosa, so that vitamin A plus an oily fish diet is needed to rescue remaining cones in this condition.

Taking into account this relationship between rods and cones and the fact that most forms of retinitis pigmentosa result in a final common pathway of predominant loss of rods among remaining cones, we can explain why vitamin A benefits so many patients with retinitis pigmentosa even though they are losing vision from different gene defects. Vitamin E appears to inhibit the absorption and transport of vitamin A resulting in decreased vitamin A levels in serum and presumably the retina as well; this provides an explanation for the adverse effect of vitamin E on this condition (Berson et al., 1993).

6 Studies of Animal Models of Retinal Degenerations

To understand and treat human retinal degenerations, a generally accepted plan is to find the causes of these diseases, produce these diseases in animal models, treat the animal models, and, where possible, conduct controlled clinical trials to determine

whether a disease can be reversed, stabilized, or slowed in affected humans. Another therapeutic strategy is to replace the missing or abnormal photoreceptor cells or pigment epithelial cells or induce other cells in the retina to substitute for photoreceptors. Another approach is an epidemiological one; risk factor analyses of well-defined populations are performed in search of ameliorating or aggravating factors as a lead in determining possible treatments for these conditions.

Nutritional intervention with vitamin A has been evaluated in transgenic mice with rhodopsin gene defects. Specifically, mice with rhodopsin, T17M, fed a high vitamin A diet for 4 months, were found to have an outer nuclear layer that was twice as thick as that of mice fed a standard diet (Fig. 5). Furthermore, the ERG in a T17M mouse raised on a high vitamin A diet was twice as large at four months compared with the ERG from a T17M mouse on a standard diet. Stated simply, high vitamin A in the diet saved retinal structure and function in this model, and these results support the observations in humans with retinitis pigmentosa. This effect was not seen in mice with rhodopsin, P347S (Li et al., 1998). In the clinical trial of vitamins A and E, 44 patients were subsequently found to have rhodopsin mutations and vitamin A was found, on average, to have a therapeutic effect in this subgroup as monitored by the ERG, but there were insufficient patients to subdivide further this subgroup into class I (e.g. P347S) and class II (e.g. T17M) mutations. Subtracting the patients with rhodopsin mutations from the entire data set in the trial of vitamins A and E, the treatment effect of vitamin A was still significant at the $p=0.01$ level. Furthermore, in every subgroup analysis performed in this trial (i.e. different genetic types, older versus younger) the trend was toward benefit, so it does not appear that any one subgroup was driving the overall treatment effect of vitamin A for retinitis pigmentosa.

With respect to gene therapy, research on a canine model with an inherited RPE65 deficiency and retinal degeneration has shown that this inherited abnormality can be treated by single dose rAAV-mediated transfer of the RPE65 gene in the retina, setting a precedent for a similar attempt at therapy in humans with this gene

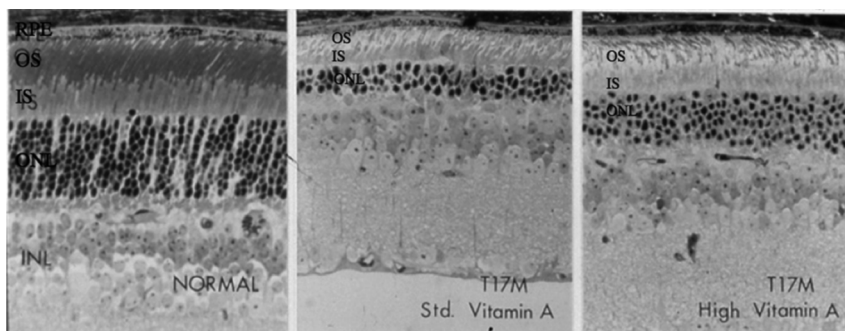


Fig. 5 Mice with a rhodopsin, T17M mutation on a high vitamin A diet for 4 months (right photo) have an outer nuclear layer (ONL) that is twice as thick as that of T17M mice on a standard vitamin A diet (middle photo). The retina from a normal mouse on a standard diet is illustrated for comparison (left photo). (Modified from Li et al., *Proc Natl Acad Sci USA*, 95:11936, 1998)

defect and Leber congenital amaurosis (Acland et al., 2005). Some of the genes known to cause retinitis pigmentosa have been destroyed or “knocked-out” in mice and these mice have then developed retinal degenerations. For example, knock-out of the RPGRIP gene in mice causes severe photoreceptor degeneration (Fig. 6, middle) as observed in humans with this gene defect that causes another form of Leber congenital amaurosis (Pawlyk et al., 2005). The RPGRIP knock-out or “KO” mouse has been treated by injecting a RPGRIP gene under the retina at age 1 month. At age 4 months the KO mouse that received gene therapy (Fig. 6, upper right) shows that the outer nuclear layer (ONL), containing primarily rod photoreceptors, is twice as thick as that in the KO control mouse (Fig. 6, upper middle). Comparing the lower right photograph with the lower middle photograph in Fig. 6, one can see that gene therapy has also resulted in a restoration of the organized layered structure of the photoreceptor outer segments (designated by the symbol OS) compared to the disorganization seen in the KO control mouse (Pawlyk et al., 2005). Work is in progress

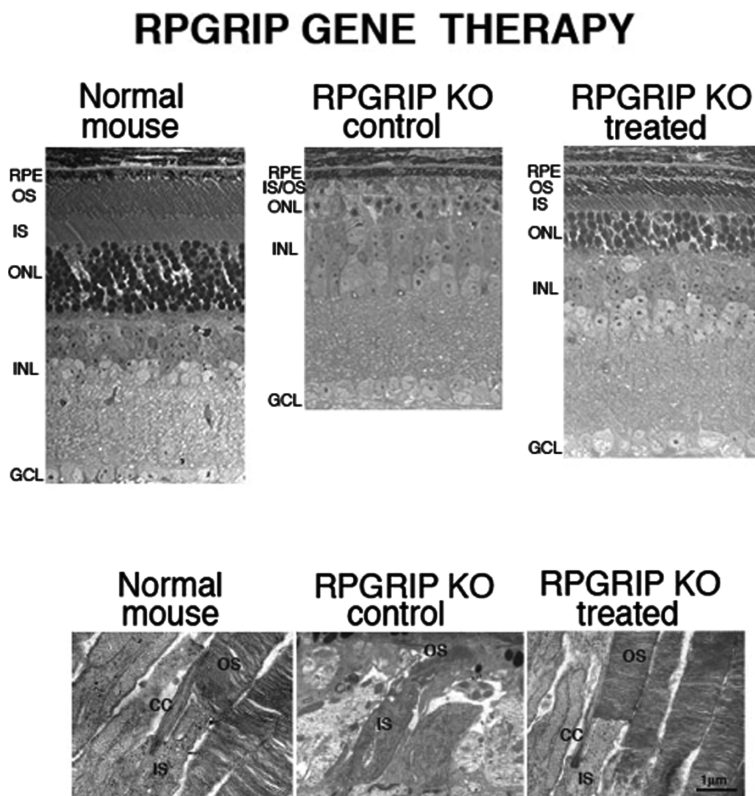


Fig. 6 Gene replacement therapy rescues photoreceptor generation in a knockout (KO) murine model of Leber congenital amaurosis lacking RPGRIP (i.e. retinitis pigmentosa GTP-ase regulator interacting protein). (Modified from Pawlyk et al., *Invest Ophthalmol Vis Sci*, 46:3042, 2005)

Table 3 Treatment strategies in animal models of retinal degenerations

(1)	Gene replacement therapy (for recessive gene mutations)
(2)	Ribozyme-based or RNAi based therapy (for dominant gene mutations)
(3)	Nutritional interventions
(4)	Neurotrophic or growth factors
(5)	Treatments with small molecules (e.g. aminoglycosides)
(6)	Light deprivation
(7)	Stem cell therapy
(8)	Inhibitors of apoptosis (e.g. caspase inhibitors)
(9)	Channel blockers
(10)	Retinal and or RPE transplantation
(11)	Non-viral gene therapy using nanoparticle technology

to see if cones can be rescued in this animal model in preparation for a trial of gene therapy for photoreceptors in children with this form of Leber congenital amaurosis.

Some treatment strategies for animal models of retinal degenerations are listed (Table 3) (Hartong et al., 2006). Gene replacement for recessive mutations and RNAi-based therapy, sometimes referred to as “gene silencing”, for dominant mutations are of current interest. Nutritional interventions should continue to be explored. Neurotrophic factors, small molecule therapies such as aminoglycosides, and stem cell therapy have precedent of success in treating animal models or human disease. Light deprivation and inhibitors of apoptosis also deserve further study in animal models with known gene abnormalities. Channel blockers such as D-cis-diltiazem have so far not been shown to be beneficial. Retina and/or retinal pigment epithelial cell transplantation are also of theoretical interest and therefore deserve research. Nanoparticle technology has been recently described as a non-viral approach for gene transfer to ocular tissues (Farjo et al., 2006). Extending some of these studies to humans presents considerable challenges as any treatment modality proposed must not only be effective but must also be safe. In the case of gene therapy, it may be difficult to introduce genes under the retina in retinitis pigmentosa because of adhesions of the retina to the pigment epithelium in more advanced stages of this condition; moreover this approach may not prove effective in patients who no longer have rod or cone photoreceptor cells to accept gene transfection.

7 Future Treatments for Human Retinal Degenerations

With respect to the future for human retinal degenerations, some imminent new therapies hold promise for different retinal degenerations. A trial of lutein, zeaxanthine, and DHA supplementation is being considered for age-related macular degeneration. Light deprivation and pharmacologic interruption of the vitamin A cycle are possible treatments for Stargardt disease. Gene therapy is being considered for children with early stages of severe forms of retinitis pigmentosa, such as Leber congenital amaurosis and Usher syndrome, type IB, as well as for Stargardt disease. Studies in animal models and a phase I study in humans support the proposal that

neuroprotective agents such as ciliary neurotrophic factor (CNTF) may stabilize retinitis pigmentosa (Sieving et al., 2006).

Other proposed therapies include optical devices to allow patients improved mobility despite constricted fields and visual prosthetic devices (such as the light-sensitive microchip) for advanced degenerations. Stem cell mediated therapy is being considered for macular degeneration or retinitis pigmentosa. It may also be possible to replace defective retinal pigment epithelium with transplanted normal pigment epithelium or stem cells and thereby benefit some patients with these conditions. For patients with advanced photoreceptor degeneration, studies are underway in an animal model of advanced disease to see if ganglion cells can be induced through gene transfection to produce melanopsin and thereby respond to light and transmit information to the brain that would allow perception of forms (Shang et al., 2007). Risk factor analyses combined with molecular genetic findings may reveal additional factors that could slow the course of these conditions with possible implications for therapy. Clearly much work remains to be done and the opportunities for rational investigations are enormous.

The scientific progress already made should encourage more investigators to enter the field of retinal degenerations. Many opportunities exist for rational laboratory studies particularly since animal models of human conditions can now be created by gene targeting manipulations. The expansion of knowledge with respect to causes and pathogenesis has created new opportunities for therapeutic interventions. Collaborations between scientists and clinicians should enhance the likelihood of developing new treatments for these diseases.

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