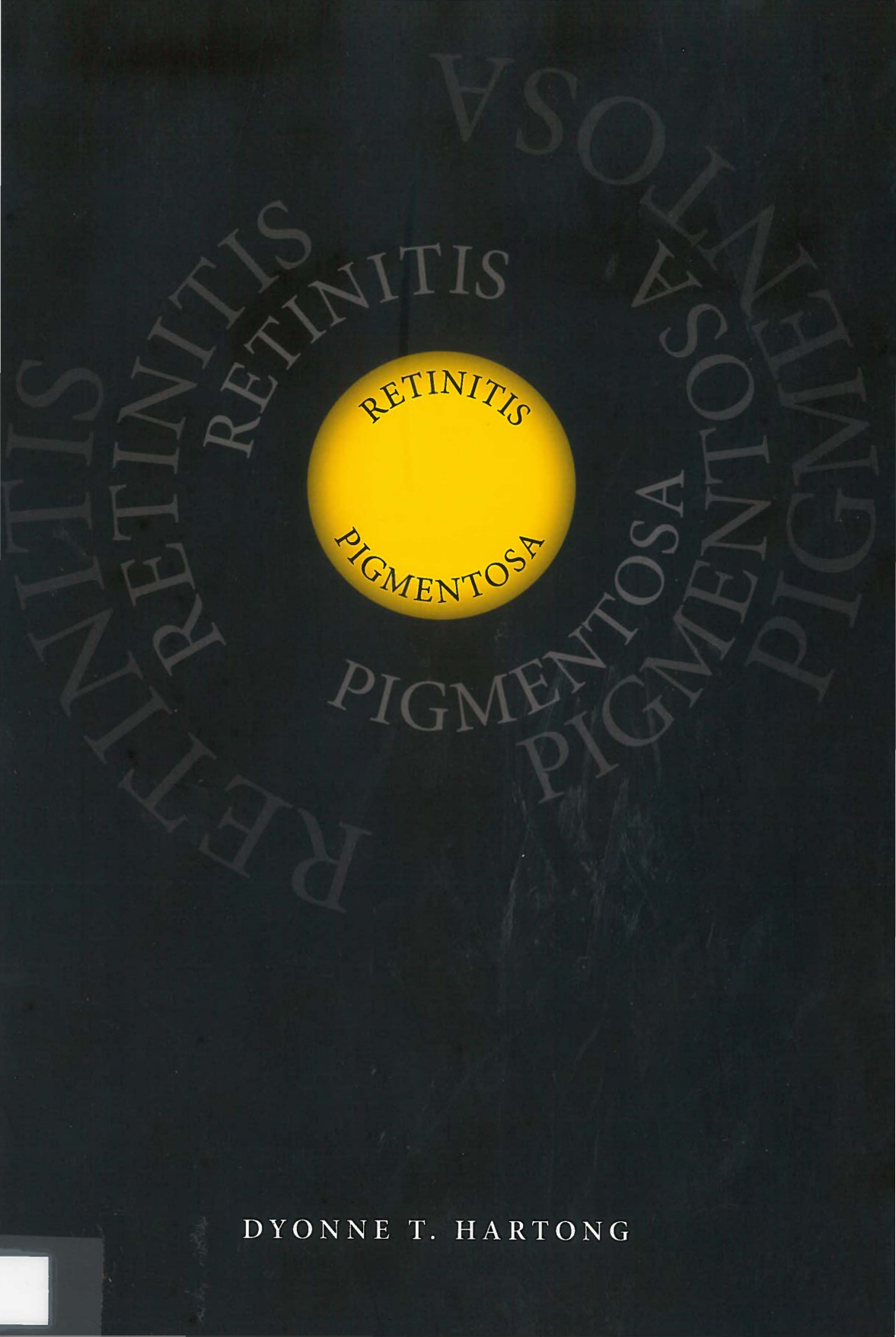


DYONNE T. HARTONG

RETINITIS PIGMENTOSA

DYONNE T. HARTONG

Retinitis pigmentosa (RP) is a degenerative disorder typically affecting the retinal rod photoreceptors first, resulting in night-blindness, and progressive deterioration of the cones, often ending in blindness. The disease has a prevalence of about 1 in 4000 and considers a major cause of visual impairment under the age of 60. The discovery of multiple disease-causing genes starting from 1990 has contributed to an increasing knowledge of the retinal function. However, the genetic heterogeneity still makes it a complex disease with respect to the understanding of common underlying processes and the resulting lack of curative therapy. It is estimated that the multiple contributing genes identified so far represent 60 - 70% of the total number of genes that cause RP. The identification of remaining genes that cause RP or hereditary retinal diseases, as well as the identification of other factors that contribute to the severity of disease, remains important in order to extend our knowledge of the retinal processes. Although some developments with respect to therapy seem encouraging for the future, we must not forget today's patient. Symptoms of night blindness, tunnel vision, and low visual acuity may be helped by vision aids and adaptation strategies, usually provided by visual rehabilitation institutes.

Retinitis Pigmentosa

Dyonne T. Hartong

STELLINGEN

Behorende bij het proefschrift

"Retinitis pigmentosa"

door Dyonne T. Hartong

1. NADP afhankelijke isocitraat dehydrogenase speelt een belangrijkere rol in de citroenzuurcyclus dan het NAD afhankelijke isocitraat dehydrogenase met mogelijk uitzondering van de retina (dit proefschrift).

2. Micro-array mRNA expressie analyse kan gebruikt worden ter opsporing van recessief overgeërfde genmutaties (dit proefschrift).

3. Individuele verschillen in telomeerlengte lijken geen verklaring voor het verschil in de ernst van retinitis pigmentosa bij patiënten met eenzelfde genmutatie (dit proefschrift).

4. Bradyopsie moet worden toegevoegd aan de differentiaal diagnose van patiënten met klachten van fotofobie en onverklaarde wisselende visusmetingen welke verbeteren bij gebruik van de stenopeische opening (dit proefschrift).

5. De nachtzichtbril geeft een toename van onafhankelijkheid en kan worden toegepast in het dagelijks leven van retinitis pigmentosa patiënten (dit proefschrift).

6. Retinitis pigmentosa patiënten moeten actief verwezen worden naar een visueel revalidatiecentrum (dit proefschrift).

7. Vuurwerk is oogverblindend.

8. Het Fenway Park in Boston is een van de weinige parken in de wereld zonder bomen.

9. Er komt steeds meer bewijs dat de Nederlandse uitdrukking 'Elkaar niet kunnen luchten of zien' waar is voor wat de voorkeur van vrouwen voor 'Luchten' van mannen aangaat (Joep Hartong).

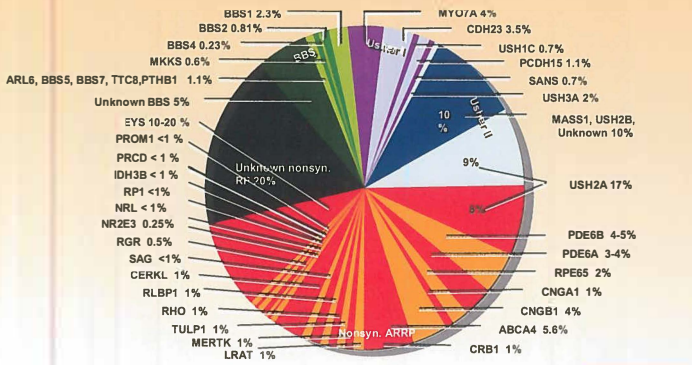
10. Een toppunt van gehandicapten voorziening betreft de aanwezigheid van braille op informatieborden op uitkijkpunten van het Empire State Building.

11. Interne kinderopvang is een vereiste om continue topklinische zorg te waarborgen.

12. De TomTom heeft op de gebruiker een vergelijkbaar effect als eerder de calculator heeft opgewekt.

13. Evolution took time, so does the unravelling of it.

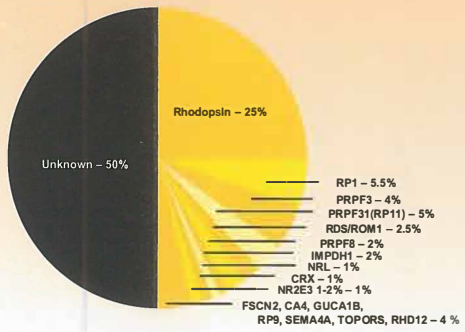
Recessive 50 – 60%



5-6 % ABCA4 !!!!! Vit A

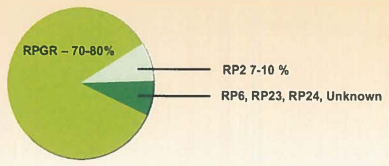
20 % USH2A

Dominant 30 - 40 %



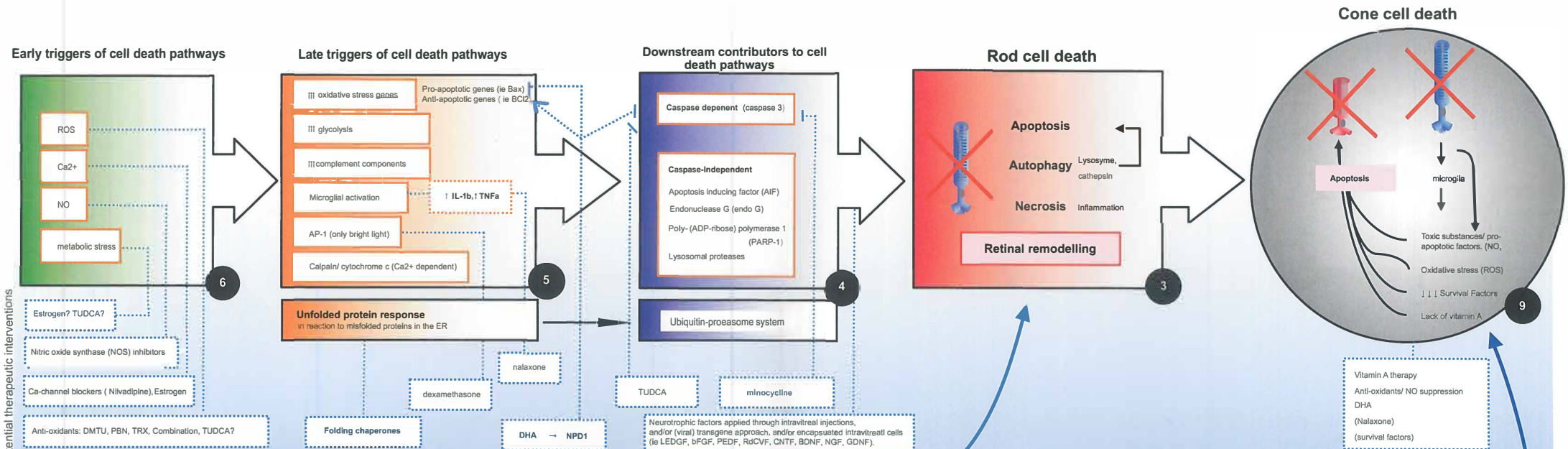
25 % RHO

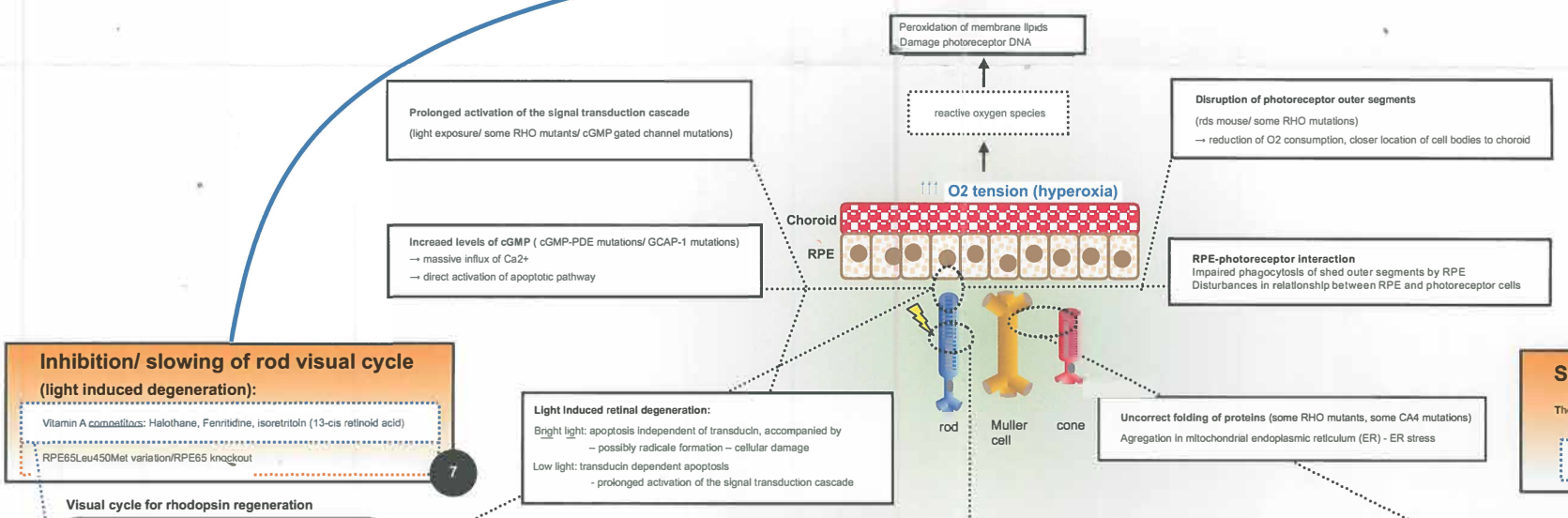
X-linked 10 -15 %



70 % RPGR

Genes and their relative contribution to retinitis pigmentosa 1





Inhibition/ slowing of rod visual cycle (light induced degeneration):

Vitamin A competitors: Halothane, Fenritidine, isoretritoin (13-cis retinoid acid)

RPE65Leu450Met variation/RPE65 knockout

7

Stimulation of cone? visual cycle:

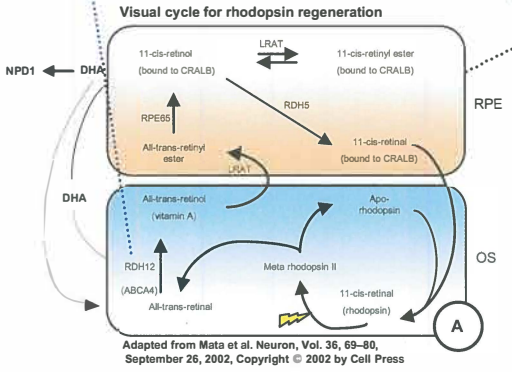
Theory: rods give atROL(vitamin A) to Muller cells and thus support the cones

Vitamin A supplementation

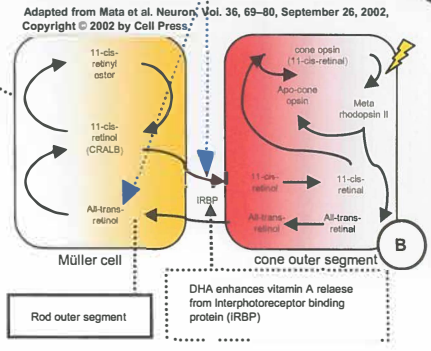
DHA from omega-3 rich fish

8

Visual cycle for rhodopsin regeneration



Adapted from Mata et al. Neuron, Vol. 36, 69–80, September 26, 2002, Copyright © 2002 by Cell Press



Completed clinical trials:

Positive study:

Vitamin A supplementation

Omega-3 rich fish

DHA capsule supplementation at 1st two years of vitamin A therapy

Uncertain outcome:

Alpha2-agonist (brimonidine)

Lutein supplementation

Fetal retina + RPE transplant

Hyperbaric oxygen therapy

Negative study:

DHA capsule supplementation after two years of vitamin A therapy

Ongoing clinical trials:

Supplementary:

DHA

Vitamin A

Anti-oxidants (lutein, zeaxanthin, alpha lipoic acid, reduced l-glutathione)

Gene/ implant/ transplant:

Electrical stimulation

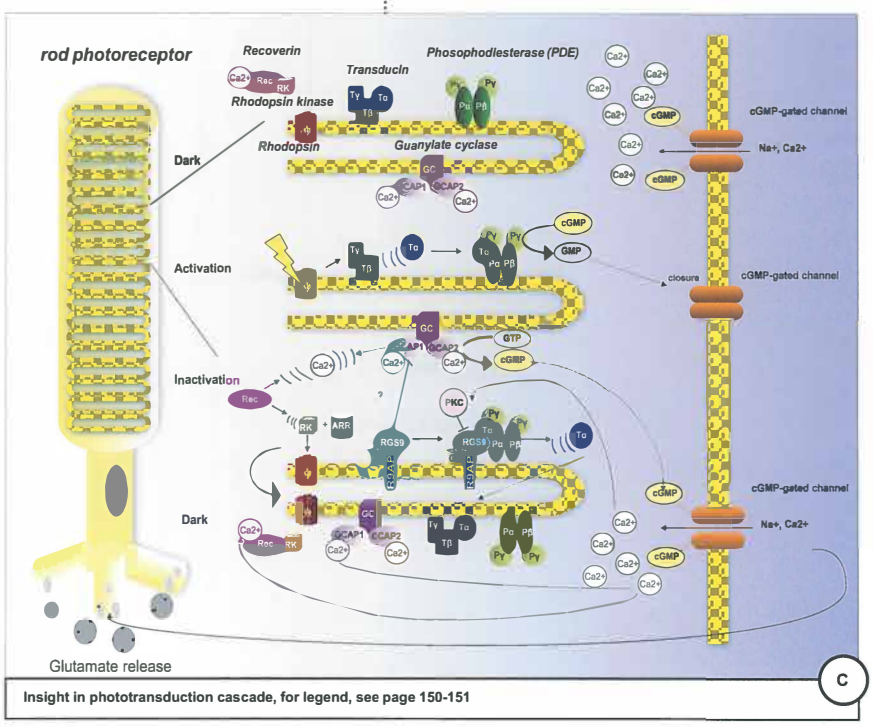
RPE 65 gene transfer

Fetal retina + RPE transplant

Pharmacological:

0.5% cyclosporine A

Alpha2-agonist – intravitreal



Insight in phototransduction cascade, for legend, see page 150-151

Gene therapy
Gene replacement
Gene silencing (ribozyme, RNAi)
Splice site correction (U1 snRNA)
Exon skipping)

Transplantation retina/RPE

Electrical stimulation

2

Thesis: Retinitis Pigmentosa, 2009
Dyonne T. Hartong
University of Groningen, Groningen, The Netherlands

ERRATUM

Behorende bij het proefschrift 'Retinitis Pigmentosa'

door Dyonne T. Hartong

aan de Rijksuniversiteit Groningen

Als gevolg van een conversie tussen verschillende tekstbestanden is er een fout opgetreden in de volgorde van de referenties nr. 67 t/m 264 behorende bij hoofdstuk 1 'Retinitis Pigmentosa' van het proefschrift.

De juiste referenties kunt u vinden in het online artikel dat beschikbaar is via www.pubmed.com: Lancet. 2006; 368:1795-809. PMID: 17113430

of

voor de gehele aangepaste versie van het proefschrift kunt u ter zijner tijd terecht bij de elektronische bibliotheek van de Rijksuniversiteit Groningen: http://dissertations.ub.rug.nl

The research presented in this thesis was supported by ZonMw-InZicht grant 943-01-011, National Eye Institute grants EY08683 and EY00169 and EY014104, US National Institutes of Health grants NIH-HL67774, CA082838 and CA132190, and the Foundation Fighting Blindness, Owings Mills, MD, USA, Retina Nederland.

Publication of this thesis was financially supported by: Alcon Netherlands B.V., Abbott Medical Optics Netherlands B.V., Allergan B.V., Becton Dickinson B.V. BD Medical-Ophthalmic Systems, Carl Zeiss B.V., Dutch Ophthalmic Research Center International B.V., Landelijke Stichting voor Blinden en Slechtzienden, Laméris Ootech B.V., Merck Sharp & Dohme B.V., Novartis Pharma B.V., Oculenti B.V., Prof. Mulder Stichting, Rijksuniversiteit Groningen, Rotterdamse vereniging blindenbelangen, Thea Pharma B.V., Tramedico B.V.

ISBN 978-90-9024259-0

Typesetting and layout: Hartong ontwerp/uitwerkstudio BV, Hilversum, NL
Printed by: Gildeprint, Enschede, NL

rijksuniversiteit groningen

Retinitis Pigmentosa

Proefschrift

ter verkrijging van het doctoraat in de
Medische Wetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, dr. F. Zwarts,
in het openbaar te verdedigen op
woensdag 17 juni 2009
om 14.45 uur

door

Dyonne Tabitha Hartong
Geboren op 20 juli 1977
te Naarden

Promotores:	Prof. dr. A.C. Kooijman Prof. dr. J.M.M. Hooymans Prof. dr. T.P. Dryja
Beoordelingscommissie:	Prof. dr. C. Wijmenga Prof. Dr. G.H.M.B van Rens Prof. Dr. A. A. Bergen

Paranimfen:	M. Smilde - Koops D. Croonen

Aan mijn ouders

Voor Folkert en Babette

Table of contents

General introduction

Retinitis pigmentosa (RP) is a degenerative disorder typically affecting the rod photoreceptors first, resulting in night-blindness, and progressive deterioration of the cones, often ending in blindness. The disease has a prevalence of about 1 in 4000 and considers a major cause of visual impairment under the age of 60. The discovery of multiple disease-causing genes starting from 1990 has contributed to an increasing knowledge of the retinal function. However, the genetic heterogeneity still makes it a complex disease with respect to the understanding of common underlying processes and the resulting lack of curative therapy. It is estimated that the 55 contributing genes (including Bardet-Biedl and Usher syndrome) identified so far represent 60-70% of the total number of genes that cause RP. The identification of remaining genes that cause RP, as well as the identification of genes that cause other hereditary retinal diseases remains important in order to extend our knowledge of the retinal processes. Chapter 1 of this thesis considers a review of the disease describing epidemiology, symptoms, diagnosis, and a summary of all known genetic causes so far. Chapter 2 describes the identification of a novel gene defect that causes RP using micro-array messenger ribonucleic acid (mRNA) expression analysis, a novel approach for the search of recessive mutations.

The identification of other factors, beside the specific gene defect, that contribute to the disease process may also provide us clues with respect to interventional strategies. Chapter 3 describes the hypothesis test that chromosomal telomere length (repetitive elements at the end of chromosomes, previously associated with cell death and age-related diseases) is associated with disease severity in RP.

Chapter 4 is not actually about RP, but it describes a non-progressive hereditary retinal disease, bradyopsia, of which the clinical findings are very important to the general knowledge of retinal function.

Although some developments, as outlined in chapter 1 and chapter 7 seem encouraging for the future, we must not forget today's patient. Symptoms of night blindness, tunnel vision, and low visual acuity may be helped by vision aids and adaptation strategies, usually provided by visual rehabilitation institutes. Symptoms may already present from young adolescence and usually impair independent (night time) activities throughout most of the patient's life. Accurate tests and implementation of vision aids that address the patient's needs are therefore warranted. Chapter 5 and 6 describe the application of night-vision goggles and its effect on subjective independence for patients with RP.

Chapter 7 discusses future perspectives with respect to expectations of therapy with the purpose to provide the reader a realistic perspective of potential treatment options for this complex disease.

Chapter 1

Retinitis pigmentosa

Dyonne T Hartong [a], Eliot L Berson [b] and Thaddeus P Dryja [a]

[a]Ocular Molecular Genetics Institute, Harvard Medical School, Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston, MA 02114, USA
[b]Berman-Gund Laboratory for the Study of Retinal Degenerations, Harvard Medical School, Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston, MA 02114, USA

Lancet. 2006 Nov 18;368(9549):1795-809.

Summary

Hereditary degenerations of the human retina are genetically heterogeneous, with well over 100 genes implicated so far. This Seminar focuses on the subset of diseases called retinitis pigmentosa, in which patients typically lose night vision in adolescence, side vision in young adulthood, and central vision in later life because of progressive loss of rod and cone photoreceptor cells. Measures of retinal function, such as the electroretinogram, show that photoreceptor function is diminished generally many years before symptomic night blindness, visual-field scotomas, or decreased visual acuity arise. More than 45 genes for retinitis pigmentosa have been identified. These genes account for only about 60% of all patients; the remainder have defects in as yet unidentified genes. Findings of controlled trials indicate that nutritional interventions, including vitamin A palmitate and omega-3-rich fish, slow progression of disease in many patients. Imminent treatments for retinitis pigmentosa are greatly anticipated, especially for genetically defined subsets of patients, because of newly identified genes, growing knowledge of affected biochemical pathways, and development of animal models.

Retinitis pigmentosa is the term given to a set of hereditary retinal diseases that feature degeneration of rod and cone photoreceptors. This Seminar will review the current status of our knowledge of this disorder, including its prevalence and inheritance patterns, symptoms and signs, molecular genetics, current treatments, and anticipated future treatment approaches.

Prevalence and inheritance patterns

The worldwide prevalence of retinitis pigmentosa is about 1 in 4000 for a total of more than 1 million affected individuals. The disease can be inherited as an autosomal-dominant (about 30–40% of cases), autosomal-recessive (50–60%), or X-linked (5–15%) trait.[1, 2 and 3] These proportions for inheritance patterns assume that all isolated cases – ie, patients with no other affected relatives – are autosomal recessive, although a few might represent new dominant mutations, instances of uniparental isodisomy,[4 and 5] or, for males, X-linked mutations. Non-mendelian inheritance patterns, such as digenic inheritance and maternal (mitochondrial) inheritance, have been reported but probably account for only a small proportion of cases.[6, 7, 8, 9 and 10] In a multicentre study from Japan including 29 vision rehabilitation centres, retinitis pigmentosa was the major cause of visual handicap or blindness, accounting for 25% of patients.[11] In Kuwait, this disease was the leading cause of visual disability in individuals younger than 60 years,[12] and in Denmark, retinitis pigmentosa and optic neuropathy were the

leading causes of blindness in people aged 20–64 years, each accounting for 29% of cases.[13]

Syndromic retinitis pigmentosa

Retinitis pigmentosa is a disease usually confined to the eye. However, some 20–30% of patients have associated non-ocular disease, and such cases fall within more than 30 different syndromes.
Usher's syndrome, in which retinitis pigmentosa is associated with hearing impairment, is the most frequent syndromic form, accounting for about 20–40%[14] of individuals with recessive disease (or 10–20% of all cases). The hearing loss can be either profound, present at birth, and associated with vestibular ataxia (Usher's syndrome type I) or moderate to mild in severity and non-progressive (type II). Normal hearing can be present in youth but during later years gradual hearing loss can occur (type III). Alterations in at least 11 genes cause Usher's syndrome; different mutations in some of these genes lead to type I, II, or III disease.[15] Depending on the mutation, some genes for Usher's syndrome can also cause either retinitis pigmentosa without hearing loss[4, 16 and 17] or deafness without retinitis pigmentosa.[18, 19, 20, 21 and 22]

Another major form of syndromic retinitis pigmentosa is Bardet-Biedl syndrome, in which retinitis pigmentosa is variably associated with obesity, cognitive impairment, polydactyly, hypogenitalism, and renal disease (mostly structural abnormalities such as calyceal cysts or calyceal clubbing and blunting);[23 and 24] some patients develop renal failure and need transplantation. Bardet-Biedl syndrome accounts for as many as 5–6% of cases of retinitis pigmentosa.[25 and 26] Ten genes for Bardet-Biedl syndrome have been identified, which cause about 70% of cases.[27, 28 and 29] Inheritance is generally a mendelian autosomal-recessive pattern; however, in some families, mutations at two unlinked Bardet-Biedl genes have been recorded,[8 and 30] with compound heterozygosity (or homozygosity) present at one locus and one mutation at the second.[8, 29, 30 and 31] Whether the mutation at the second locus is needed to express the disease or whether it merely modifies severity or expressivity of mutations at the other locus is still unclear. The proportion of Bardet-Biedl families showing digenic inheritance might be low.[32]

Of the many rare syndromic forms of retinitis pigmentosa, three are important clinically. In these disorders, treatment might be vision-saving if begun early: abetalipoproteinaemia (Bassen-Kornzweig syndrome); phytanic acid oxidase deficiency (Refsum's disease); and familial isolated vitamin E deficiency (α tocopherol transport protein deficiency).[33]

Symptoms

Retinitis pigmentosa is a highly variable disorder; some patients develop symptomatic visual loss in childhood whereas others remain asymptomatic until mid-adulthood. Many patients fall into a classic pattern of difficulties with dark adaptation and night blindness in adolescence and loss of mid-peripheral visual field in young adulthood. As the disease advances they lose far peripheral vision, eventually develop tunnel vision, and finally lose central vision, usually by age 60 years.

Visual symptoms indicate the gradual loss of the two photoreceptor types (figure 1): rods, which mediate achromatic vision in starlight or moonlight; and cones, which are important for colour vision and fine acuity in daylight. The outer nuclear layer of the retina consists of rod and cone photoreceptor nuclei and is severely attenuated in patients with retinitis pigmentosa. The inner nuclear layer—composed of amacrine cell, bipolar cell, and horizontal cell neurons—and the ganglion-cell layer are fairly well preserved, but many of these cells degenerate later in the disease.

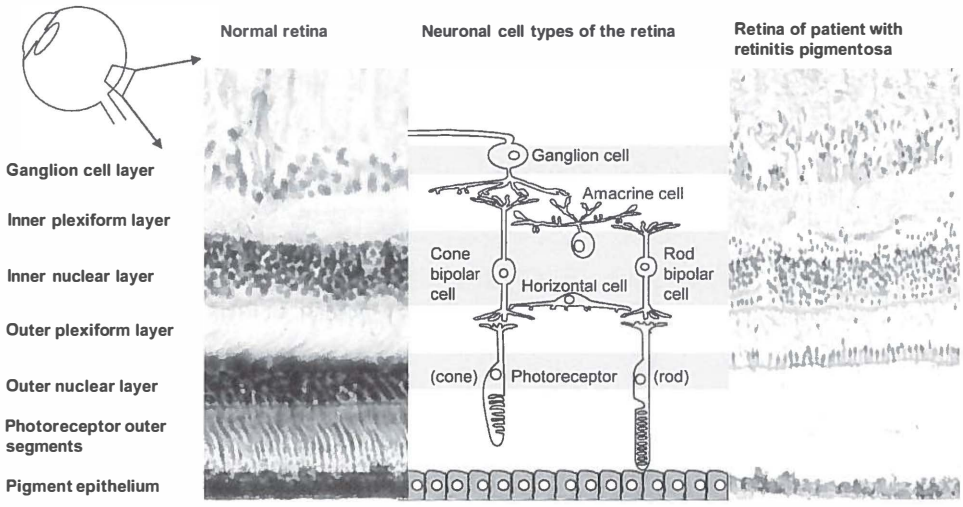


Figure 1. Histological appearance of healthy human retina (left) and retina of a patient with retinitis pigmentosa at a mid-stage of disease (right). The space between the retinal pigment epithelium and the outer nuclear layer in the diseased retina is a processing artifact.

Most patients are legally blind by age 40 years because of severely constricted visual fields. In most forms of typical retinitis pigmentosa, loss of rod function exceeds reduction of cone sensitivity. In other types, rod and cone decline is similar.

Occasionally, the deficit of cones far exceeds that of rods, which is termed cone-rod degeneration,[34] a form of retinitis pigmentosa in which loss of visual acuity and defective colour vision are the prominent early symptoms.
A clinician must be cautious when relying on symptoms to identify patients with early retinitis pigmentosa. In our electrically illuminated night-time environment, people can be unaware of a severe loss of rod function because night-time activities are typically done with sufficient light to allow vision with cones. By the time an individual recognises the symptom of night blindness, a reduction in cone sensitivity can have happened on top of a loss of rod function. Furthermore, no subjective difficulties with daily tasks may arise in people with a remaining central visual field reduced to about 50 degrees in diameter (normal bilateral visual field is about 180 degrees in the horizontal meridian).[35] Patients can lose 90% of cones in the fovea before having a reduction in visual acuity.[36] Reading impairment and difficulties in undertaking daily activities are typically seen when visual acuities fall below 0·5 (20/40).[37 and 38] Objective measures of photoreceptor sensitivity (see below) are much more reliable than symptoms for diagnosis of retinitis pigmentosa and grading its severity.

Clinical assessment and findings

Visual acuity can remain normal even in individuals with advanced retinitis pigmentosa with a small island of remaining central visual field, or it can be lost early in the course of the disorder. Neglect of careful determination of refractive errors in people with severe visual loss can happen, yet patients can be very grateful for the modest improvement in vision that spectacles might provide. Furthermore, a measure of refractive error could give a clue to the inheritance pattern. For example, patients with X-linked retinitis pigmentosa are likely to have myopia of 2 dioptres or more, whereas hyperopia favours a diagnosis of dominant inheritance.[39 and 40]

Visual fields, measured with a Goldmann perimeter or a Humphrey field analyser (Carl Zeiss, Dublin, CA, USA), typically have scotomas in the mid-periphery that enlarge over years owing to loss of rod and cone function. In moderate-to-advanced retinitis pigmentosa, only small islands of vision remain in the far peripheral field and in the visual axis; later these areas of vision slowly disappear.

Colour vision assessed with Ishihara plates, the Farnsworth D15 panel (Munsell Colour Laboratory; Macbeth, New Windsor, NY, USA), or other tests might show normal colour vision or a deficiency in blue cone function (acquired tritanopia), which is characteristic of advanced retinitis pigmentosa. If a red or green colour

deficiency is present, a diagnosis of an anomaly in colour vision—eg, X-linked colour blindness present in 5–8% of all males—or cone-rod or cone degeneration should be considered.
The final dark adaptation threshold is a measure of the degree of night blindness under moonlight and starlight conditions. It is measured after the patient adapts to 30 min of darkness with eye patches or by being in a completely dark room. The lowest intensity of white light that is able to be perceived is then measured. If this intensity is at least 100 times brighter than normal (ie, the final dark adaptation threshold is raised 2 log units or more), a severe loss of rod photoreceptor sensitivity has arisen and individuals should be cautioned about driving at dusk or at night irrespective of the status of their visual acuity or visual fields. Large increases in threshold indicate a decrease in cone photoreceptor sensitivity as well.

Contrast sensitivity is measured with a contrast chart (ie, Pelli-Robson chart).[41] A decline in contrast sensitivity is a common finding in patients with retinitis pigmentosa,[42] and it can account for poor subjective vision in those people who have good high contrast visual acuity.[43]

Slit-lamp biomicroscopy and ophthalmoscopy show posterior subcapsular cataracts in about 50% of individuals with retinitis pigmentosa.[39, 44, 45 and 46] Cells in the vitreous are commonly seen. Attenuation of retinal vessels is an almost universal finding (figure 2). The fundus typically shows intraretinal pigmentation, sometimes referred to as bone-spicule deposits because of their shape, in the mid-periphery or far periphery (figure 2). They might be absent, especially early in the course of disease.[39] Pigment deposits are created when the retinal pigment epithelium (a pigmented cell layer adjacent to photoreceptors) migrates into the neural retina in response to photoreceptor-cell death.[47] The optic nerve head can have a waxy pale colour (figure 2).

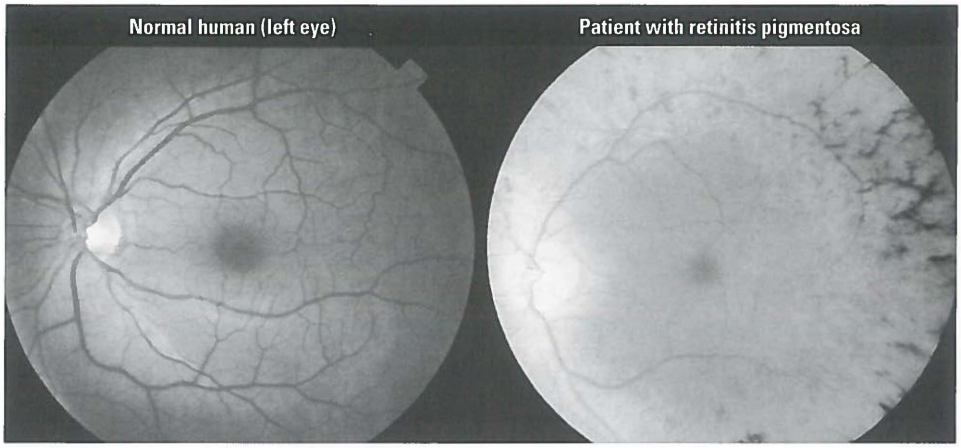


Figure 2. Fundi of a healthy individual (left) and a patient with retinitis pigmentosa (right).

In the image of the diseased eye, optic-disc pallor, attenuated retinal arterioles, and peripheral intraretinal pigment deposits in a bone-spicule configuration are seen.

Electroretinograms (ERGs) measure the electrical response of the retina to flashes of light and are recorded with either a contact-lens electrode on the topically anaesthetised cornea or an electrode applied to the eyelid. A single-flash dim blue light elicits a rod response, a brighter single-flash white light elicits a combined rod-plus-cone response, and flickering (30 Hz) white light stimuli generate cone-isolated responses (figure 3). With single flashes (0·5 Hz) of white light, an initial a wave shows hyperpolarisation of photoreceptors and a subsequent b wave results from depolarisation of cells in the inner nuclear layer. Patients with retinitis pigmentosa have reduced rod and cone response amplitudes and a delay in their timing (figure 3).[48 and 49] Amplitudes of the a and b waves can be either moderately reduced (as in dominant disease) or almost non-detectable (as seen in recessive and X-linked patients). Time intervals from stimuli to peak rod or cone isolated responses are prolonged in typical retinitis pigmentosa. ERG amplitudes are objective measures of retinal function and are useful for accurate diagnosis of disease, for assessment of severity,[50 and 51] to follow the course of disease,[52 and 53] to provide a visual prognosis,[53] and for measurement of responses to treatments.[53] With conventional recordings without computer averaging, most patients have non-detectable full-field cone response amplitudes (<10 μV; normal ≥50 μV) even when they have substantial cone vision; with computer averaging, ERG sensitivity is extended 100-fold. Patients with cone ERG amplitudes as low as 1 μV or less can still have ambulatory vision and read newspapers; most people with amplitudes less than 0·05 μV are legally blind or have only light perception.[54]

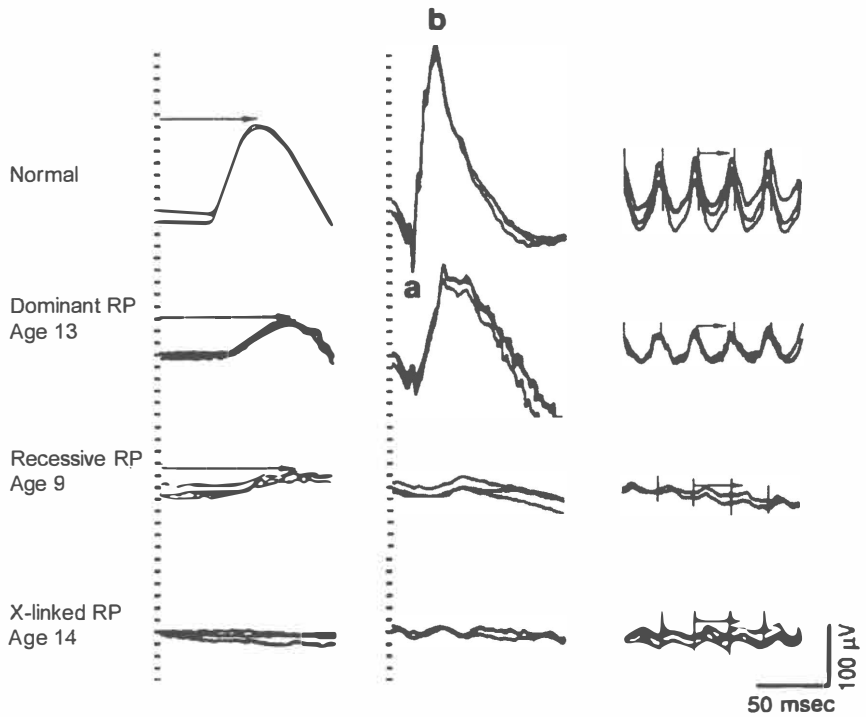


Figure 3. ERG responses from a healthy individual and from three patients with early retinitis pigmentosa inherited as an autosomal-dominant, autosomal-recessive, or X-linked trait.
RP=retinitis pigmentosa. a=a wave. b=b wave. Vertical dotted lines (left and centre columns) and vertical shock artifacts (right column) represent stimuli. Arrows indicate response times (called implicit times).

Optical coherence tomography is a non-invasive technique for assessment of the morphology of the retina and particularly of the macula. It is especially useful in patients with retinitis pigmentosa for measurement of retina thickness, assessment of the status of the photoreceptor layer, and determining the presence of macular oedema.[55, 56, 57 and 58]

Images of fundus autofluorescence show that some patients with retinitis pigmentosa have raised concentrations of lipofuscin in retinal pigment epithelium. Regions of the retina with the highest amounts of autofluorescence are those producing the lowest ERG amplitudes, as measured with multifocal ERGs.[59 and 60]

Course of retinitis pigmentosa

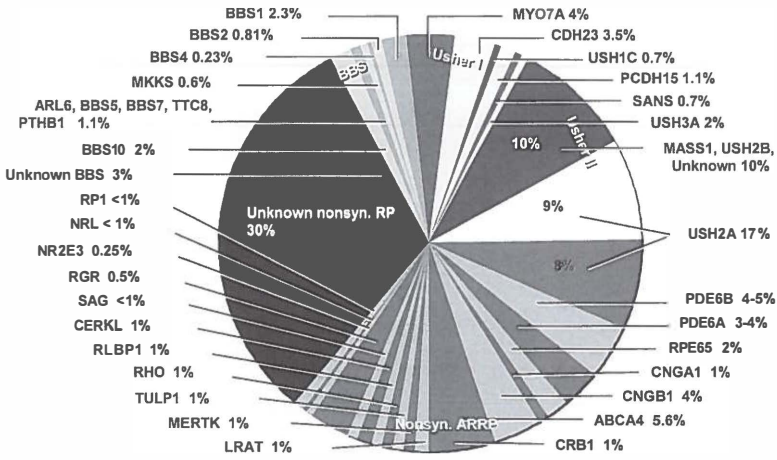
The age of onset of retinitis pigmentosa typically refers to the age at which a patient reports visual symptoms, and it can range from early childhood to adulthood.

Because of the striking variation in how aware individuals are of their visual loss, the age of onset of symptoms is an imprecise measure of disease severity, and it gives little or no indication of when photoreceptor degeneration actually begins. ERGs and other tests show that photoreceptor degeneration is already present as early as age 6 years, even in patients who remain asymptomatic until young adulthood.[61] Clinical examinations, especially those including objective quantitative measures of retinal function, are crucial to describe accurately the degree of visual compromise and rate of its decline. This information is necessary to give a prognosis for vision customised to every patient. Individuals older than age 6 years with normal ERGs have not been reported to develop typical retinitis pigmentosa at a later time.[61]

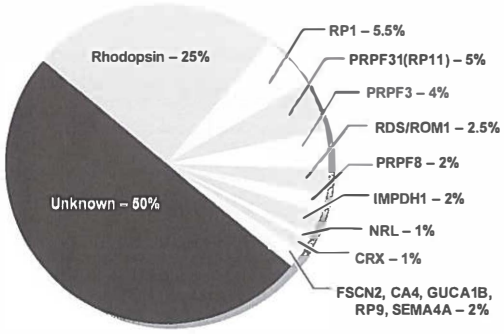
In general, retinitis pigmentosa is a progressive disease with an apparently exponential decline[62] in remaining visual-field area (2·6–13·5% loss annually)[34, 63 and 64] and ERG amplitude (8·7–18·5%).[34, 63 and 65] Variations in reported rates of decline have been attributed to stage of disease, environmental and dietary factors, primary gene defects, and possible modifier genes. Visual acuity better than 0·1 (20/200) reflects the function of foveal cones and, since the fovea is generally the last region of the retina to deteriorate, good acuity can persist for many years in patients with only tiny islands of remaining peripheral visual field and very low ERG amplitudes.[52] Thus, clinical trials and studies to monitor progression of disease usually include visual fields and ERG amplitudes. However, subjective visual handicap correlates best with visual acuity and less well with visual field and ERG amplitudes.[66]

Causal genes

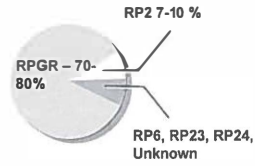
Most cases of retinitis pigmentosa are monogenic, but the disease is nevertheless very heterogeneous genetically. Investigators have identified at least 45 loci so far at which mutations cause the disorder, and these genes collectively account for disease in a little over half of all patients (figure 4). Most genes for retinitis pigmentosa cause only a small proportion of cases (figure 4), exceptions being the rhodopsin gene (*RHO*), which leads to about 25% of dominant retinitis pigmentosa, the *USH2A* gene, which might cause about 20% of recessive disease (including many with Usher's syndrome type II), and the *RPGR* gene that accounts for about 70% of X-linked retinitis pigmentosa. In aggregate, mutations in *RHO*, *USH2A*, and *RPGR* genes cause about 30% of all cases of retinitis pigmentosa.



A) Autosomal recessive retinitis pigmentosa, 50 - 60% of cases



B) Autosomal dominant retinitis pigmentosa, 30 - 40% of cases



C) X-linked retinitis pigmentosa, 5 - 15% of cases

Figure 4. Genes and their relative contribution to retinitis pigmentosa.

Causal genes and their contributions to (A) autosomal-recessive disease (ARRP), including Usher's and Bardet-Biedl (BBS) syndromes, (B) autosomal-dominant disease, and (C) X-linked disease. About 40% of cases are due to genes that are as yet undiscovered. In A, these cases are represented by three pie slices named unknown non-syndromic retinitis pigmentosa 30%; unknown BBS 3%; and part of MASS1, USH2B, and unknown 10%. All digenic cases with RDS/ROM1 mutations are included in the dominant category. The figure does not include Leber congenital amaurosis, cone-rod dystrophy, macular degeneration, or cases with maternal inheritance (eg, Kearns-Sayre syndrome). For some genes, only one or a few families have been reported with mutations; in these cases, we have arbitrarily set the gene frequency at 1%. Our estimates for the proportions of cases accounted for by every gene are based on data from the following articles. Autosomal-recessive retinitis pigmentosa: ABCA4;[67] CERKL;[68] CNGA1;[69] CNGB1;[70] CRB1;[71] LRAT;[72] MERTK;[73] NR2E3;[74 and 75] NRL;[76] PDE6A;[77] PDE6B;[78 and 79] RGR;[80] RHO;[81 and 82] RLBP1;[83 and 84] RP1;[85 and 86] RPE65;[87] SAG;[88] TULP1;[89] USH2A;[90 and 91] Bardet-Biedl syndrome;[27, 29 and 92] Usher's syndrome type I;[93] USH3A.[94] Autosomal-dominant disease: RHO;[67, 69, 95, 96 and 97] RP1;[97 and 98] PRPF31 (unpublished data from the authors); PRPF3 (unpublished data from the authors); RDS/ROM1;[99] PRPF8 (unpublished data from the authors); IMPDH1;[100 and 101] NRL;[102] CRX;[97 and 103] CA4(RP17);[104] FSCN2;[105] GUCA1B;[106] RP9;[107] SEMA4A.[108] X-linked disease: RPGR and RP2;[109 and 110] RP6, RP23, and RP24 are mapped to X chromosome but remain unidentified.[111, 112 and 113]

Affected biochemical pathways

The table categorises currently identified genes for retinitis pigmentosa according to the known or presumed function of the encoded proteins. Some of the genes normally encode proteins in the rod photoreceptor cascade, a specific biochemical pathway that transduces light and leads to changes in photoreceptor-cell polarisation. Recessive null mutations in any of these genes would evidently interfere with rod function and produce night blindness from birth. Subsequent death of rod photoreceptors is probably an outcome of the deranged physiology associated with the defective or absent gene product. For example, without functional rod cGMP-phosphodiesterase, which arises with recessive defects in *PDE6A* or *PDE6B*, cGMP concentrations in photoreceptor outer segments rise, which in turn opens an excessive proportion of cGMP-gated cation channels in the plasma membrane.[164, 165 and 166] Rods apparently die from the rush of cations flowing into the cell through these open channels. As another example, dominant rhodopsin mutations are probably detrimental to rods because the mutant forms of rhodopsin are toxic to rod photoreceptors. The toxic effects are attributable to interference with metabolism, perhaps by formation of intracellular protein aggregates, from a defect in intracellular transport, or from a fault in the structure of the photoreceptor outer segments.[167, 168, 169, 170, 171, 172 and 173]

	Inheritance
Phototransduction cascade	
RHO, rhodopsin (G-protein coupled photon receptor)[114]	Dominant, recessive
PDE6A, rod cGMP-phosphodiesterase α subunit (G-protein effector enzyme)[115 and 116]	Recessive
PDE6B, rod cGMP-phosphodiesterase α subunit (G-protein effector enzyme)[115 and 116]	Recessive
CNGA1, rod cGMP-gated cation channel α subunit[117]	Recessive
CNGB1, rod cGMP-gated cation channel α subunit[118, 119 and 120]	Recessive
SAG, arrestin (rhodopsin deactivation)[121]	Recessive
Vitamin A metabolism	
ABCA4, ATP-binding cassette protein A4 (photoreceptor disc membrane flippase for vitamin A)[122 and 123]	Recessive
RLBP1, retinaldehyde binding protein (11-cis-retinaldehyde carrier)[124]	Recessive
RPE65, (vitamin A trans-cis isomerase)[125 and 126]	Recessive
LRAT, lecithin retinol acetyltransferase (synthesises vitamin A esters)[125]	Recessive
RGR, RPE-vitamin A G-protein coupled receptor (photon receptor in RPE)[127]	Recessive
Structural or cytoskeletal	
RDS, peripherin (outer disc segment membrane protein)[128 and 129]	Dominant, digenic
ROM1, rod outer segment protein[130]	Digenic
FSCN2, fascin (actin bundling protein)[131 and 132]	Dominant
TULP1, tubby-like protein 1[133]	Recessive
CRB1, crumbs homologue (transmembrane protein, adherent junctions)[134]	Recessive
RP1, microtubule-associated protein (microtubule formation and stabilisation)[135]	Dominant, recessive
Signalling, cell-cell interaction, or synaptic interaction	
SEMA4A, semaphorin B, transmembrane immune system protein[136]	Dominant
CDH23, cadherin 23 (adhesion receptor)[137 and 138]	Recessive
PCDH15, protocadherin 15 (adhesion receptor)[139]	Recessive
USH1C, Usher's syndrome type 1C (integrating scaffold protein harmonin)[140]	Recessive
USH2A, Usher's syndrome type IIA (Usher's network protein)[140]	Recessive
MASS1, monogenic audiogenic seizure susceptibility 1 (Usher's network protein)[140]	Recessive
USH3A, Usher's syndrome type IIIA (transmembrane protein clarin 1)[141]	Recessive
RP2, plasma membrane associated protein[142]	X-linked
RNA intron-splicing factors	
PRPF31, precursor mRNA-processing factor 31 (spliceosome component)[143]	Dominant
PRPF8, precursor mRNA-processing factor 8 (spliceosome component)[144]	Dominant
PRPF3, precursor mRNA-processing factor 3 (spliceosome component)[145 and 146]	Dominant
RP9, PIM1-associated protein (RNA splicing factor)[147]	Dominant

	Inheritance
Trafficking of intracellular proteins	
MYO7A, myosin 7A (melanosome motility protein)[148]	Recessive
USH1G, scaffold protein containing ankyrin repeats and SAM domain (Usher's type I protein traffic regulator)[149]	Recessive
Maintenance of cilia/ciliated cells (possible role in intracellular trafficking)	
BBS1, Bardet-Biedl syndrome 1[150]	Recessive
BBS2, Bardet-Biedl syndrome 2[150, 151 and 152]	Recessive
ARL6, ADP-ribosylation factor like 6[150]	Recessive
BBS4, Bardet-Biedl syndrome 4[150 and 153]	Recessive
BBS5, Bardet-Biedl syndrome 5[150 and 154]	Recessive
MKKS, McKusick-Kaufman syndrome[150 and 155]	Recessive
BBS7, Bardet-Biedl syndrome 7[150 and 156]	Recessive
TTC8, tetratricopeptide repeat domain 8[150, 156 and 157]	Recessive
PTHB1, parathyroid hormone-responsive B1 gene[150]	Recessive
RPGR, trafficking of proteins in the cilia [158 and 159]	X-linked
pH regulation (choriocapillaris)	
CA4, carbonic anhydrase IV (carbon dioxide/bicarbonate balance)[160]	Dominant
Phagocytosis	
MERTK, mer tyrosine kinase proto-oncogene (RPE receptor involved in outer segment phagocytosis)[161]	Recessive
Other	
CERKL, ceramide kinase-like (ceramide converting enzyme)[162]	Recessive
IMPDH1, inosine-5' monophosphate dehydrogenase type I (guanine nucleotide synthesis)[163]	Dominant
BBS10, vertebrate-specific chaperonin-like protein[29]	Recessive

RPE=retinal pigment epithelium.

Why do mutations in genes that are exclusively expressed in rod photoreceptors cause the death of both rod and cone cells? The secondary death of cones might indicate their as yet unexplained reliance on neighbouring rods for survival. Understanding the interaction between rods and cones, and the factors from rods that promote cone survival, might provide clues to treatments.[174 and 175]

Some genes for retinitis pigmentosa are expressed in tissues outside the eye, and some encode proteins that are essential for life. For example, the dominant genes *PRPF31*, *PRPF8*, and *PRPF3* encode components of the spliceosome, a vital complex that excises introns from RNA transcripts. These proteins are highly conserved in eukaryotes ranging from mammals to yeast, so the fact that mutations in these factors lead to retinitis pigmentosa without other evidence of systemic disease in patients is especially fascinating.

Treatment

Based on a study of the natural course of retinitis pigmentosa,[63] patients who happen to be taking vitamin A, vitamin E, or both were recorded to have slower declines in ERG amplitudes than those not taking such supplements.[53] This observation prompted a randomised clinical trial of oral vitamin A and E supplements in 601 patients with dominant, recessive, and X-linked non-syndromic retinitis pigmentosa and Usher's syndrome type II.[53] Participants were randomly assigned either daily vitamin A as retinyl palmitate 15 000 IU, vitamin E 400 IU as *dl*-α-tocopherol, the combination, or trace amounts of both vitamins; follow-up was for 4–6 years. Patients assigned high-dose vitamin A showed a significantly ($p=0·01$) slower decline in cone ERG amplitudes than did those in the other groups. Differences were more pronounced ($p<0·001$) in a subgroup of 354 individuals with higher initial cone ERG amplitudes; in these people, a significant ($p=0·04$) negative effect of vitamin E was also recorded.[53]

Critics of the trial pointed out that measures of retinal function other than cone ERG—such as visual-field area and visual acuity—did not differ significantly between groups,[176] and that results with cone ERGs were of only modest significance.[177] However, visual-field area has substantial inter-visit variability, so that a small change in the decline of visual-field area would probably not have been detectable with the study design. In a subsequent analysis of 125 participants who did visual-field tests with the greatest precision (≤5% inter-visit variability), those assigned vitamin A showed a significantly slower loss of field than did those not taking vitamin A.[178 and 179] Furthermore, in most patients, visual acuity declines slowly or not at all in earlier stages,[180] and thus to note a therapeutic effect would

need a larger or longer study than was undertaken. As far as we are aware, no clinical trials by other groups to assess the effectiveness of vitamin A supplements have been undertaken.

Based on these results, many clinicians recommend that adults with early or middle stages of retinitis pigmentosa take 15 000 IU of oral vitamin A palmitate every day and avoid high-dose vitamin E supplements. β carotene is not a suitable substitute for vitamin A because it is not reliably converted to vitamin A. People on this regimen should have annual measurements of fasting vitamin A concentrations in serum and liver function, although no cases of toxic effects have been reported.[181] Older individuals should also be monitored for bone health because a slight increased risk for hip fractures from osteoporosis has been reported in postmenopausal women and men older than 49 years who take vitamin A supplements.[182 and 183] Because of an enhanced risk for birth defects, high-dose vitamin A supplements are not recommended for women who are pregnant or planning to conceive.[184] No children younger than age 18 years were included in the study, nor were people with less common forms of retinal degeneration (eg, cone-rod degeneration, Leber congenital amaurosis, and many syndromic forms of retinitis pigmentosa), and thus no formal recommendation can be made for them about vitamins A and E.

Another nutritional treatment assessed for patients with retinitis pigmentosa is docosahexaenoic acid (DHA), an omega-3 fatty acid found in high concentrations in oily fish such as salmon, tuna, mackerel, herring, and sardines. DHA is apparently important for photoreceptor function, since membranes containing rhodopsin and cone opsins in photoreceptor cells have very high concentrations of this fatty acid.[185] Amounts of DHA in red-blood cells are on average lower in patients with retinitis pigmentosa than in unaffected people, but whether the difference is attributable to a speculative metabolic variation or to changes in diet or other factors is unknown.[186 and 187] Results from two independent studies of oral DHA supplements for individuals with retinitis pigmentosa, one consisting of 44 males with X-linked disease and the other of 208 patients with various inheritance patterns, did not show a clear benefit for the treatment based on the original outcome measures.[186 and 188] However, in both studies, people with the highest concentrations of DHA in red-blood cells (combining patients on supplements and controls who possibly had high amounts from their diet) had the slowest rates of retinal degeneration.[186 and 189] Furthermore, analysis of the control group in the larger study—ie, 110 participants receiving vitamin A and placebo—showed that those with a diet containing at least 1·4 g of omega-3 fatty acids per week (equivalent to two 90 g servings of oily fish per week) lost visual field at a rate 40–50% slower than those eating less omega-3 fatty acids. Possibly, if the slower rate of degeneration were sustained for a long period,

the combined benefit of vitamin A and oily fish could provide almost 20 additional years of visual preservation for the average patient who starts this regimen in their mid-30s.[189] Some clinicians, therefore, recommend that adults with typical retinitis pigmentosa should follow this regimen.

Patients with three rare syndromic forms of retinitis pigmentosa can also benefit from specific dietary modification and nutritional supplements. First, individuals with abetalipoproteinaemia (Bassen-Kornzweig disease) have low concentrations of apolipoprotein B in plasma and have fat malabsorption, which results in low amounts in plasma of fat-soluble vitamins. Besides retinitis pigmentosa, patients develop ataxia, peripheral neuropathy, and steatorrhoea. High oral doses of vitamin A result in acute restoration of retinal function in the early stages of the disease.[190 and 191] Addition of vitamin E has been reported to stabilise the disorder.[192] Second, phytanic acid oxidase deficiency (Refsum's disease) is associated with cardiac conduction defects, ataxia, polyneuropathy, deafness, anosmia, dry skin, and retinitis pigmentosa. Dietary modification to severely reduce intake of phytanic acid while maintaining bodyweight can slow or stop progression of this form of retinitis pigmentosa.[193] Finally, familial isolated vitamin E deficiency (α tocopherol transport protein deficiency) can cause adult-onset ataxia, dysarthria, reduced touch and position sense, and retinitis pigmentosa. Treatment with vitamin E has been reported to halt progression of this disease.[194]

Reduction in exposure to light is postulated to be beneficial for patients with retinitis pigmentosa. This hypothesis is lent support by findings in two animal models of the disease (both with rhodopsin mutations), in which constant darkness was associated with a reduction in the rate of degeneration[195] or in which brief exposures to bright light hastened loss of photoreceptors.[196] Two patients (one later found to have digenic retinitis pigmentosa with mutations in the *RDS* and *ROM1* genes)[99] tested the effect of light deprivation on their retinitis pigmentosa by occluding one eye for 6 h per day for 5 years.[197] No difference in the extent of retinal degeneration was recorded between occluded and unoccluded eyes. Separately, an individual with retinitis pigmentosa had a monocular occlusion of the pupil from childhood trauma, causing more than a tenfold reduction in light to the retina; the pupil was surgically opened 40 years later, yet the traumatised eye had a funduscopic appearance and ERGs equivalent to the fellow eye.[198] As far as we know, no studies of light exposure with many patients, either prospective or retrospective, have been undertaken. The benefit of modulation of light exposure for individuals with certain genetically defined forms of retinitis pigmentosa remains to be established.

Some measures do not directly benefit the retina but nevertheless help patients with vision loss related to retinitis pigmentosa. Cataract extraction is indicated in individuals with lens opacities that substantially reduce distance and near vision. Carbonic anhydrase inhibitors can provide transient improvement in visual acuity in people with oedema of the macula.[199 and 200] Patients should be encouraged to visit vision-rehabilitation clinics, at which (for example) a night vision pocket scope or goggles[201 and 202] or a wide-angle mobility lamp[203] could be offered to improve night vision. Hand-held and computer magnification devices could boost reading vision in individuals with advanced disease.

The future

With knowledge of causal genes in more than half of patients with retinitis pigmentosa, and increasing knowledge about associated biochemical defects, many clinicians are optimistic that novel treatments for the disorder will soon be developed. Many mechanistically diverse approaches to treat retinitis pigmentosa are being investigated. These include: 1) gene-specific approaches; 2) interventions in secondary biochemical pathways that could benefit groups of patients with various gene defects; 3) transplantation to replace lost retinal tissue; and 4) implanted electrical devices.

Gene-therapy approaches are dependent on the type of mutation. Recessively inherited diseases typically result from alterations that eliminate the encoded protein (loss-of-function mutations). For this type of genetic change, introduction of a normal copy of the gene into the diseased tissue (gene-replacement treatment) might induce local production of the missing protein. One notable gene-replacement approach to a form of retinitis pigmentosa is on the verge of human trials. The target gene is *RPE65*, which encodes the isomerase in the retinal pigment epithelium that is essential for production of the photopigment 11-cis-retinal. In patients and animal models without this enzyme owing to recessive *RPE65* mutations, many photoreceptors survive for a long time after severe visual loss.[55, 204 and 205] By transiently providing 11-cis-retinal or a related photopigment pharmacologically, these cells are seen to be functional.[55 and 206] A window of opportunity is therefore available during which replacement of the *RPE65* gene might restore vision. Subretinal injection of adeno-associated virus vectors containing the *RPE65* gene has shown success in restoring vision in mice and dogs with mutations in *RPE65*.[204, 207, 208, 209, 210, 211 and 212] Gene-replacement treatment has also been successful in animal models of other genetically identified forms of retinitis pigmentosa,[213, 214, 215, 216, 217 and 218] but many of the approaches will not be easily transferred to human beings. One difficulty is that many patients have

already lost all or nearly all rod photoreceptors and are hoping for a treatment to save the few remaining cone photoreceptors. Techniques such as optical coherence tomography will be valuable adjuncts in clinical trials since they can provide a measure of the status of the photoreceptor cell layer and establish whether patients with vision loss have cells available for rescue.[57, 58 and 204]

Dominantly inherited mutations typically alter the transcribed aminoacid sequence and result in toxic variants of the encoded protein (termed gain-of-function mutations). One strategy to treat these alterations is to eliminate the mutant gene (gene silencing) and hope that the remaining normal copy of the gene will provide sufficient functional protein. Current experimental approaches to accomplish this aim include ribozyme-based or interference RNA (RNAi)-based gene therapy to inactivate or reduce expression of specific dominant alleles.[219, 220, 221, 222 and 223]

Nutritional or neuroprotective treatments or approaches that affect secondary biochemical pathways have the advantage of being less dependent on the disease-causing mutation than genetic strategies and could therefore be widely applicable—eg, treatment might interfere with apoptosis.[224, 225, 226, 227 and 228] Findings of work done in animals have shown that some neurotrophic factors can promote photoreceptor survival.[174, 175, 229, 230, 231 and 232] Results of a human phase I study of an intravitreal capsule containing cells that release ciliary neurotrophic factor have been reported.[233] Of some concern, one patient in the study had a decline in ERG amplitudes; however, the same individual and some others had improvements in visual acuity over the 6-month duration of the study.

Small-molecule drugs are also being assessed as possible treatments for forms of retinitis pigmentosa. For example, in a study of a calcium-channel blocker (diltiazem), researchers claimed a beneficial effect in a mouse model of a form of recessive retinitis pigmentosa due to recessive mutations in the β subunit of rod phosphodiesterase.[234] However, three subsequent trials of this drug in mice and other animal models by independent groups failed to confirm a benefit.[235, 236 and 237]

Many research groups are studying the potential value of transplantation of the retinal pigment epithelium,[238, 239, 240, 241 and 242] photoreceptors,[243] or stem cells.[244, 245, 246, 247, 248, 249, 250 and 251] Results of transplantation of retinal pigment epithelium have shown a slight increase in visual acuity in one patient;[252] a phase II clinical trial is ongoing. Stem cells have been shown to differentiate into cells that express retina-specific markers.[244, 245, 246 and 247] Embryonic stem cells transplanted in rats and mice integrate into the host retina[248 and 249] and seem to protect host retinal neurons.[248]

Devices to electrically stimulate the retina, optic nerve, or visual cortex are being developed and tested in animal models and patients.[253, 254, 255, 256, 257, 258, 259 and 260] The few people tested with the first versions of these devices have reported seeing phosphenes (flashes of light) in response to direct retinal stimulation.[261, 262, 263 and 264]

In view of the growing research effort on therapeutic approaches for retinitis pigmentosa, new treatments for some forms of the disease will probably be helping subsets of patients within the next 5–10 years. Strategies to save or restore vision in all individuals might need many decades of research.

References

1 Bunker CH, Berson EL, Bromley WC, Hayes RP, Roderick TH. Prevalence of retinitis pigmentosa in Maine. *Am J Ophthalmol* 1984; 97: 357-65.

2 Grondahl J. Estimation of prognosis and prevalence of retinitis pigmentosa and Usher syndrome in Norway. *Clin Genet* 1987; 31: 255-64.

3 Novak-Lauš K, Suzana Kukulj S, Zoric-Geber M, Bastaic O. Primary tapetoretinal dystrophies as the cause of blindness and impaired vision in the republic of Croatia. *Acta Clin Croat* 2002; 41: 23-7.

4 Rivolta C, Berson EL, Dryja TP. Paternal uniparental heterodisomy with partial isodisomy of chromosome 1 in a patient with retinitis pigmentosa without hearing loss and a missense mutation in the Usher syndrome type II gene USH2A. *Arch Ophthalmol* 2002; 120: 1566-71.

5 Thompson DA, McHenry CL, Li Y, Richards JE, Othman MI, Schwinger E, Vollrath D, Jacobson SG, Gal A. Retinal dystrophy due to paternal isodisomy for chromosome 1 or chromosome 2, with homoallelism for mutations in RPE65 or MERTK, respectively. *Am J Hum Genet* 2002; 70: 224-9.

6 Mansergh FC, Millington-Ward S, Kennan A, Kiang AS, Humphries M, Farrar GJ, Humphries P, Kenna PF. Retinitis pigmentosa and progressive sensorineural hearing loss caused by a C12258A mutation in the mitochondrial MTTS2 gene. *Am J Hum Genet* 1999; 64: 971-85.

7 Kajiwara K, Berson EL, Dryja TP. Digenic retinitis pigmentosa due to mutations at the unlinked peripherin/RDS and ROM1 loci. *Science* 1994; 264: 1604-8.

8 Katsanis N, Ansley SJ, Badano JL, Eichers ER, Lewis RA, Hoskins BE, Scambler PJ, Davidson WS, Beales PL, Lupski JR. Triallelic inheritance in Bardet-Biedl syndrome, a Mendelian recessive disorder. *Science* 2001; 293: 2256-9.

9 Lestienne P, Ponsot G. Kearns-Sayre syndrome with muscle mitochondrial DNA deletion. *Lancet* 1988; 1: 885.

10 Zeviani M, Moraes CT, DiMauro S, Nakase H, Bonilla E, Schon EA, Rowland LP. Deletions of mitochondrial DNA in Kearns-Sayre syndrome. *Neurology* 1988; 51: 1525.

11 Hata H, Yonezawa M, Nakanishi T, Ri T, Yanashima K. Causes of entering institutions for visually handicapped persons during the past fifteen years. *Jpn J Clin Ophthalmol* 2003; 57: 259-62.

12 Al Merjan JI, Pandova MG, Al Ghanim M, Al Wayel A, Al Mutairi S. Registered blindness and low vision in Kuwait. *Ophthalmic Epidemiol* 2005; 12: 251-7.

13 Buch H, Vinding T, La Cour M, Appleyard M, Jensen GB, Nielsen NV. Prevalence and causes of visual impairment and blindness among 9980 Scandinavian adults: the Copenhagen City Eye Study. *Ophthalmology* 2004; 111: 53-61.

14 Boughman JA, Vernon M, Shaver KA. Usher syndrome: definition and estimate of prevalence from two high-risk populations. *J Chronic Dis* 1983; 36: 595-603.

15 Pennings RJ, Fields RR, Huygen PL, Deutman AF, Kimberling WJ, Cremers CW. Usher syndrome type III can mimic other types of Usher syndrome. *Ann Otol Rhinol Laryngol* 2003; 112: 525-30.

16 Bernal S, Ayuso C, Antinolo G, Gimenez A, Borrego S, Trujillo MJ, Marcos I, Calaf M, Del Rio E, Baiget M. Mutations in USH2A in Spanish patients with autosomal recessive retinitis pigmentosa: high prevalence and phenotypic variation. *J Med Genet* 2003; 40: e8.

17 Aller E, Najera C, Millan JM, Oltra JS, Perez-Garrigues H, Vilela C, Navea A, Beneyto M. Genetic analysis of 2299delG and C759F mutations (USH2A) in patients with visual and/or auditory impairments. *Eur J Hum Genet* 2004; 12: 407-10.

18 Bork JM, Peters LM, Riazuddin S, Bernstein SL, Ahmed ZM, Ness SL, Polomeno R, Ramesh A, Schloss M, Srisailpathy CR, Wayne S, Bellman S, Desmukh D, Ahmed Z, Khan SN, Kaloustian VM, Li XC, Lalwani A, Riazuddin S, Bitner-Glindzicz M, Nance WE, Liu XZ, Wistow G, Smith RJ, Griffith AJ, Wilcox ER, Friedman TB, Morell RJ. Usher syndrome 1D and nonsyndromic autosomal recessive deafness DFNB12 are caused by allelic mutations of

the novel cadherin-like gene CDH23. *Am J Hum Genet* 2001; 68: 26-37.
19 Astuto LM, Bork JM, Weston MD, Askew JW, Fields RR, Orten DJ, Ohliger SJ, Riazuddin S, Morell RJ, Khan S, Riazuddin S, Kremer H, van Hauwe P, Moller CG, Cremers CW, Ayuso C, Heckenlively JR, Rohrschneider K, Spandau U, Greenberg J, Ramesar R, Reardon W, Bitoun P, Millan J, Legge R, Friedman TB, Kimberling WJ. CDH23 mutation and phenotype heterogeneity: a profile of 107 diverse families with Usher syndrome and nonsyndromic deafness. *Am J Hum Genet* 2002; 71: 262-75.
20 Liu XZ, Walsh J, Mburu P, Kendrick-Jones J, Cope MJ, Steel KP, Brown SD. Mutations in the myosin VIIA gene cause non-syndromic recessive deafness. *Nat Genet* 1997; 16: 188-90.
21 Weil D, Kussel P, Blanchard S, Levy G, Levi-Acobas F, Drira M, Ayadi H, Petit C. The autosomal recessive isolated deafness, DFNB2, and the Usher 1B syndrome are allelic defects of the myosin-VIIA gene. *Nat Genet* 1997; 16: 191-3.
22 Luijendijk MW, Van Wijk E, Bischoff AM, Krieger E, Huygen PL, Pennings RJ, Brunner HG, Cremers CW, Cremers FP, Kremer H. Identification and molecular modelling of a mutation in the motor head domain of myosin VIIA in a family with autosomal dominant hearing impairment (DFNA11). *Hum Genet* 2004; 115: 149-56.
23 Tieder M, Levy M, Gubler MC, Gagnadoux MF, Broyer M. Renal abnormalities in the Bardet-Biedl syndrome. *Int J Pediatr Nephrol* 1982; 3: 199-203.
24 Beales PL, Elcioglu N, Woolf AS, Parker D, Flinter FA. New criteria for improved diagnosis of Bardet-Biedl syndrome: results of a population survey. *J Med Genet* 1999; 36: 437-46.
25 Macrae WG. Retinitis pigmentosa in Ontario - a survey. *Birth Defects Orig Artic Ser* 1982; 18: 175-85.
26 Haim M. Epidemiology of retinitis pigmentosa in Denmark. *Acta Ophthalmol Scand Suppl* 2002; 1-34.
27 Nishimura DY, Swiderski RE, Searby CC, Berg EM, Ferguson AL, Hennekam R, Merin S, Weleber RG, Biesecker LG, Stone EM, Sheffield VC. Comparative Genomics and Gene Expression Analysis Identifies BBS9, a New Bardet-Biedl Syndrome Gene. *Am J Hum Genet* 2005; 77: 1021-33.
28 Stoetzel C, Laurier V, Faivre L, Megarbane A, Perrin-Schmitt F, Verloes A, Bonneau D, Mandel JL, Cossee M, Dollfus H. BBS8 is rarely mutated in a cohort of 128 Bardet-Biedl syndrome families. *J Hum Genet* 2006; 51: 81-4.
29 Stoetzel C, Laurier V, Davis EE, Muller J, Rix S, Badano JL, Leitch CC, Salem N, Chouery E, Corbani S, Jalk N, Vicaire S, Sarda P, Hamel C, Lacombe D, Holder M, Odent S, Holder S, Brooks AS, Elcioglu NH, Da Silva E, Rossillion B, Sigaudy S, de Ravel TJ, Alan LR, Leheup B, Verloes A, Amati-Bonneau P, Megarbane A, Poch O, Bonneau D, Beales PL, Mandel JL, Katsanis N, Dollfus H. BBS10 encodes a vertebrate-specific chaperonin-like protein and is a major BBS locus. *Nat Genet* 2006; 38: 521-4.
30 Beales PL, Badano JL, Ross AJ, Ansley SJ, Hoskins BE, Kirsten B, Mein CA, Froguel P, Scambler PJ, Lewis RA, Lupski JR, Katsanis N. Genetic interaction of BBS1 mutations with alleles at other BBS loci can result in non-Mendelian Bardet-Biedl syndrome. *Am J Hum Genet* 2003; 72: 1187-99.
31 Badano JL, Leitch CC, Ansley SJ, May-Simera H, Lawson S, Lewis RA, Beales PL, Dietz HC, Fisher S, Katsanis N. Dissection of epistasis in oligogenic Bardet-Biedl syndrome. *Nature* 2006; 439: 326-30.
32 Mykytyn K, Nishimura DY, Searby CC, Beck G, Bugge K, Haines HL, Cornier AS, Cox GF, Fulton AB, Carmi R, Iannaccone A, Jacobson SG, Weleber RG, Wright AF, Riise R, Hennekam RC, Luleci G, Berker-Karauzum S, Biesecker LG, Stone EM, Sheffield VC. Evaluation of complex inheritance involving the most common Bardet-Biedl syndrome locus (BBS1). *Am J Hum Genet* 2003; 72: 429-37.
33 Grant CA, Berson EL. Treatable forms of retinitis pigmentosa associated with systemic neurological disorders. *Int Ophthalmol Clin* 2001; 41: 103-10.

34 Birch DG, Anderson JL, Fish GE. Yearly rates of rod and cone functional loss in retinitis pigmentosa and cone-rod dystrophy. *Ophthalmology* 1999; 106: 258-68.
35 Szlyk JP, Seiple W, Fishman GA, Alexander KR, Grover S, Mahler CL. Perceived and actual performance of daily tasks: relationship to visual function tests in individuals with retinitis pigmentosa. *Ophthalmology* 2001; 108: 65-75.
36 Geller AM, Sieving PA. Assessment of foveal cone photoreceptors in Stargardt's macular dystrophy using a small dot detection task. *Vision Res* 1993; 33: 1509-24.
37 Grover S, Fishman GA, Anderson RJ, Tozatti MS, Heckenlively JR, Weleber RG, Edwards AO, Brown J, Jr. Visual acuity impairment in patients with retinitis pigmentosa at age 45 years or older. *Ophthalmology* 1999; 106: 1780-5.
38 Virgili G, Pierrottet C, Parmeggiani F, Pennino M, Giacomelli G, Steindler P, Menchini U, Orzalesi N. Reading performance in patients with retinitis pigmentosa: a study using the MNREAD charts. *Invest Ophthalmol Vis Sci* 2004; 45: 3418-24.
39 Berson EL, Rosner B, Simonoff E. Risk factors for genetic typing and detection in retinitis pigmentosa. *Am J Ophthalmol* 1980; 89: 763-75.
40 Fishman GA, Farber MD, Derlacki DJ. X-linked retinitis pigmentosa. Profile of clinical findings. *Arch Ophthalmol* 1988; 106: 369-75.
41 Pelli DG RJWA. The design of a new letter chart for measuring contrast sensitivity. *Clin Vision Sci* 1988;2:187-99. *Clinical Vision Sciences* 1988; 2: 187-99.
42 Lindberg CR, Fishman GA, Anderson RJ, Vasquez V.. Contrast sensitivity in retinitis pigmentosa. *Br J Ophthalmol* 1981; 65: 855-8.
43 Lodha N, Westall CA, Brent M, Abdolell M, Heon E. A modified protocol for the assessment of visual function in patients with retinitis pigmentosa. *Adv Exp Med Biol* 2003; 533: 49-57.
44 Heckenlively J. The frequency of posterior subcapsular cataract in the hereditary retinal degenerations. *Am J Ophthalmol* 1982; 93: 733-8.
45 Fishman GA, Anderson RJ, Lourenco P. Prevalence of posterior subcapsular lens opacities in patients with retinitis pigmentosa. *Br J Ophthalmol* 1985; 69: 263-6.
46 Pruett RC. Retinitis pigmentosa: clinical observations and correlations. *Trans Am Ophthalmol Soc* 1983; 81: 693-735.
47 Li ZY, Possin DE, Milam AH. Histopathology of bone spicule pigmentation in retinitis pigmentosa. *Ophthalmology* 1995; 102: 805-16.
48 Karpe G. Basis of clinical electroretinography. *Acta Ophthalmol Suppl* 1945; 24: 84.
49 Berson EL, Gouras P, Hoff M. Temporal aspects of the electroretinogram. *Arch Ophthalmol* 1969; 81: 207-14.
50 Iannaccone A, Rispoli E, Vingolo EM, Onori P, Steindl K, Rispoli D, Pannarale MR. Correlation between Goldmann perimetry and maximal electroretinogram response in retinitis pigmentosa. *Doc Ophthalmol* 1995; 90: 129-42.
51 Sandberg MA, Weigel-DiFranco C, Rosner B, Berson EL. The relationship between visual field size and electroretinogram amplitude in retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 1996; 37: 1693-8.
52 Holopigian K, Greenstein V, Seiple W, Carr RE. Rates of change differ among measures of visual function in patients with retinitis pigmentosa. *Ophthalmology* 1996; 103: 398-405.
53 Berson EL, Rosner B, Sandberg MA, Hayes KC, Nicholson BW, Weigel-DiFranco C, Willett W. A randomized trial of vitamin A and vitamin E supplementation for retinitis pigmentosa. *Arch Ophthalmol* 1993; 111: 761-72.
54 Andreasson SO, Sandberg MA, Berson EL. Narrow-band filtering for monitoring low-amplitude cone electroretinograms in retinitis pigmentosa. *Am J Ophthalmol* 1988; 105: 500-3.
55 Van Hooser JP, Aleman TS, He YG, Cideciyan AV, Kuksa V, Pittler SJ, Stone EM, Jacobson SG, Palczewski K. Rapid restoration of visual pigment and function with oral retinoid in a mouse model of childhood blindness. *Proc Natl Acad Sci U S A* 2000; 97: 8623-8.

56 Sandberg MA, Brockhurst RJ, Gaudio AR, Berson EL. The association between visual acuity and central retinal thickness in retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 2005; 46: 3349-54.
57 Jacobson SG, Cideciyan AV, Iannaccone A, Weleber RG, Fishman GA, Maguire AM, Affatigato LM, Bennett J, Pierce EA, Danciger M, Farber DB, Stone EM. Disease expression of RP1 mutations causing autosomal dominant retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 2000; 41: 1898-908.
58 Ko TH, Fujimoto JG, Schuman JS, Paunescu LA, Kowalevicz AM, Hartl I, Drexler W, Wollstein G, Ishikawa H, Duker JS. Comparison of ultrahigh- and standard-resolution optical coherence tomography for imaging macular pathology. *Ophthalmology* 2005; 112: 1922.
59 Robson AG, Saihan Z, Jenkins SA, Fitzke FW, Bird AC, Webster AR, Holder GE. Functional characterisation and serial imaging of abnormal fundus autofluorescence in patients with retinitis pigmentosa and normal visual acuity. *Br J Ophthalmol* 2006; 90: 472-9.
60 Popovic P, Jarc-Vidmar M, Hawlina M. Abnormal fundus autofluorescence in relation to retinal function in patients with retinitis pigmentosa. *Graefes Arch Clin Exp Ophthalmol* 2005; 243: 1018-27.
61 Berson EL. Retinitis pigmentosa. The Friedenwald Lecture. *Invest Ophthalmol Vis Sci* 1993; 34: 1659-76.
62 Clarke G, Collins RA, Leavitt BR, Andrews DF, Hayden MR, Lumsden CJ, McInnes RR. A one-hit model of cell death in inherited neuronal degenerations. *Nature* 2000; 406: 195-9.
63 Berson EL, Sandberg MA, Rosner B, Birch DG, Hanson AH. Natural course of retinitis pigmentosa over a three-year interval. *Am J Ophthalmol* 1985; 99: 240-51.
64 Grover S, Fishman GA, Anderson RJ, Alexander KR, Derlacki DJ. Rate of visual field loss in retinitis pigmentosa. *Ophthalmology* 1997; 104: 460-5.
65 Berson EL, Rosner B, Weigel-DiFranco C, Dryja TP, Sandberg MA. Disease progression in patients with dominant retinitis pigmentosa and rhodopsin mutations. *Invest Ophthalmol Vis Sci* 2002; 43: 3027-36.
66 Szlyk JP, Fishman GA, Alexander KR, Revelins BI, Derlacki DJ, Anderson RJ. Relationship between difficulty in performing daily activities and clinical measures of visual function in patients with retinitis pigmentosa. *Arch Ophthalmol* 1997; 115: 53-9.
67 Farber DB, Lolley RN. Cyclic guanosine monophosphate: elevation in degenerating photoreceptor cells of the C3H mouse retina. *Science* 1974; 186: 449-51.
68 Pittler SJ, Baehr W. Identification of a nonsense mutation in the rod photoreceptor cGMP phosphodiesterase beta-subunit gene of the rd mouse. *Proc Natl Acad Sci U S A* 1991; 88: 8322-6.
69 Bowes C, Li T, Frankel WN, Danciger M, Coffin JM, Applebury ML, Farber DB. Localization of a retroviral element within the rd gene coding for the beta subunit of cGMP phosphodiesterase. *Proc Natl Acad Sci U S A* 1993; 90: 2955-9.
70 Min KC, Zvyaga TA, Cypess AM, Sakmar TP. Characterization of mutant rhodopsins responsible for autosomal dominant retinitis pigmentosa. Mutations on the cytoplasmic surface affect transducin activation. *J Biol Chem* 1993; 268: 9400-4.
71 Sung CH, Davenport CM, Nathans J. Rhodopsin mutations responsible for autosomal dominant retinitis pigmentosa. Clustering of functional classes along the polypeptide chain. *J Biol Chem* 1993; 268: 26645-9.
72 Kaushal S, Khorana HG. Structure and function in rhodopsin. 7. Point mutations associated with autosomal dominant retinitis pigmentosa. *Biochemistry* 1994; 33: 6121-8.
73 Colley NJ, Cassill JA, Baker EK, Zuker CS. Defective intracellular transport is the molecular basis of rhodopsin-dependent dominant retinal degeneration. *Proc Natl Acad Sci U S A* 1995; 92: 3070-4.
74 Kurada P, Tonini TD, Serikaku MA, Piccini JP, O'Tousa JE. Rhodopsin maturation antagonized by dominant rhodopsin mutants. *Vis Neurosci* 1998; 15: 693-700.

75 Illing ME, Rajan RS, Bence NF, Kopito RR. A rhodopsin mutant linked to autosomal dominant retinitis pigmentosa is prone to aggregate and interacts with the ubiquitin proteasome system. *J Biol Chem* 2002; 277: 34150-60.
76 Deretic D, Williams AH, Ransom N, Morel V, Hargrave PA, Arendt A. Rhodopsin C terminus, the site of mutations causing retinal disease, regulates trafficking by binding to ADP-ribosylation factor 4 (ARF4). *Proc Natl Acad Sci U S A* 2005; 102: 3301-6.
77 Sahel JA. Saving cone cells in hereditary rod diseases: a possible role for rod-derived cone viability factor (RdCVF) therapy. *Retina* 2005; 25: S38-S39.
78 Leveillard T, Mohand-Said S, Lorentz O, Hicks D, Fintz AC, Clerin E, Simonutti M, Forster V, Cavusoglu N, Chalmel F, Dolle P, Poch O, Lambrou G, Sahel JA. Identification and characterization of rod-derived cone viability factor. *Nat Genet* 2004; 36: 755-9.
79 Massof RW, Finkelstein D. Supplemental vitamin A retards loss of ERG amplitude in retinitis pigmentosa (letter to the editor). *Arch Ophthalmol* 1993; 111: 751-4.
80 Gamel JW, Barr CC. A randomized trial of vitamin A and vitamin E supplementation for retinitis pigmentosa (letter to the editor). *Arch Ophthalmol* 1993; 111: 1462-3.
81 Berson EL. Treatment of retinitis pigmentosa with vitamin A. *Digital Journal of Ophthalmology* 2002; 4: 1-4.
82 Berson EL, Rosner B, Sandberg MA, Hayes KC, Nicholson BW, Weigel-DiFrano C, Willett W. Vitamin A supplementation for retinitis pigmentosa. *Arch Ophthalmol* 1993; 111: 1456-9.
83 Holopigian K, Greenstein V, Seiple W, Carr RE. Rates of change differ among measures of visual function in patients with retinitis pigmentosa. *Ophthalmology* 1996; 103: 398-405.
84 Sibulesky L, Hayes KC, Pronczuk A, Weigel-DiFranco C, Rosner B, Berson EL. Safety of <7500 RE (<25000 IU) vitamin A daily in adults with retinitis pigmentosa. *Am J Clin Nutr* 1999; 69: 656-63.
85 Feskanich D, Singh V, Willett WC, Colditz GA. Vitamin A intake and hip fractures among postmenopausal women. *JAMA* 2002; 287: 47-54.
86 Michaelsson K, Lithell H, Vessby B, Melhus H. Serum retinol levels and the risk of fracture. *N Engl J Med* 2003; 348: 287-94.
87 Lammer EJ, Chen DT, Hoar RM, Agnish ND, Benke PJ, Braun JT, Curry CJ, Fernhoff PM, Grix AW, Jr., Lott IT, . Retinoic acid embryopathy. *N Engl J Med* 1985; 313: 837-41.
88 Fliesler SJ, Anderson RE. Chemistry and metabolism of lipids in the vertebrate retina. *Prog Lipid Res* 1983; 22: 79-131.
89 Hoffman DR, Locke KG, Wheaton DH, Fish GE, Spencer R, Birch DG. A randomized, placebo-controlled clinical trial of docosahexaenoic acid supplementation for X-linked retinitis pigmentosa. *Am J Ophthalmol* 2004; 137: 704-18.
90 Schaefer EJ, Robins SJ, Patton GM, Sandberg MA, Weigel-DiFranco CA, Rosner B, Berson EL. Red blood cell membrane phosphatidylethanolamine fatty acid content in various forms of retinitis pigmentosa. *J Lipid Res* 1995; 36: 1427-33.
91 Berson EL, Rosner B, Sandberg MA, Weigel-DiFranco C, Moser A, Brockhurst RJ, Hayes KC, Johnson CA, Anderson EJ, Gaudio AR, Willett WC, Schaefer EJ. Clinical trial of docosahexaenoic acid in patients with retinitis pigmentosa receiving vitamin A treatment. *Arch Ophthalmol* 2004; 122: 1297-305.
92 Berson EL, Rosner B, Sandberg MA, Weigel-DiFranco C, Moser A, Brockhurst RJ, Hayes KC, Johnson CA, Anderson EJ, Gaudio AR, Willett WC, Schaefer EJ. Further evaluation of docosahexaenoic acid in patients with retinitis pigmentosa receiving vitamin A treatment: subgroup analyses. *Arch Ophthalmol* 2004; 122: 1306-14.
93 Gouras P, Carr RE, Gunkel RD. Retinitis pigmentosa in abetalipoproteinemia: Effects of vitamin A. *Invest Ophthalmol* 1971; 10: 784-93.
94 Sperling MA, Hiles DA, Kennerdell JS. Electroretinographic responses following vitamin A therapy in A-beta-lipoproteinemia. *Am J Ophthalmol* 1972; 73: 342-51.

95 Bishara S, Merin S, Cooper M, Azizi E, Delpre G, Deckelbaum RJ. Combined vitamin A and E therapy prevents retinal electrophysiological deterioration in abetalipoproteinaemia. *Br J Ophthalmol* 1982; 66: 767-70.
96 Hungerbuhler JP, Meier C, Rousselle L, Quadri P, Bogousslavsky J. Refsum's disease: management by diet and plasmapheresis. *Eur Neurol* 1985; 24: 153-9.
97 Yokota T, Shiojiri T, Gotoda T, Arita M, Arai H, Ohga T, Kanda T, Suzuki J, Imai T, Matsumoto H, Harino S, Kiyosawa M, Mizusawa H, Inoue K. Friedreich-like ataxia with retinitis pigmentosa caused by the His101Gln mutation of the alpha-tocopherol transfer protein gene. *Ann Neurol* 1997; 41: 826-32.
98 Naash ML, Peachey NS, Li ZY, Gryczan CC, Goto Y, Blanks J, Milam AH, Ripps H. Light-induced acceleration of photoreceptor degeneration in transgenic mice expressing mutant rhodopsin. *Invest Ophthalmol Vis Sci* 1996; 37: 775-82.
99 Cideciyan AV, Jacobson SG, Aleman TS, Gu D, Pearce-Kelling SE, Sumaroka A, Acland GM, Aguirre GD. In vivo dynamics of retinal injury and repair in the rhodopsin mutant dog model of human retinitis pigmentosa. *Proc Natl Acad Sci U S A* 2005; 102: 5233-8.
100 Dryja TP, Hahn LB, Kajiwara K, Berson EL. Dominant and digenic mutations in the peripherin/RDS and ROM1 genes in retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 1997; 38: 1972-82.
101 Berson EL. Light deprivation and retinitis pigmentosa. *Vision Res* 1980; 20: 1179-84.
102 Miyake Y, Sugita S, Horiguchi M, Yagasaki K. Light deprivation and retinitis pigmentosa. *Am J Ophthalmol* 1990; 110: 305-6.
103 Grover S, Fishman GA, Fiscella RG, Adelman AE. Efficacy of dorzolamide hydrochloride in the management of chronic cystoid macular edema in patients with retinitis pigmentosa. *Retina* 1997; 17: 222-31.
104 Cox SN, Hay E, Bird AC. Treatment of chronic macular edema with acetazolamide. *Arch Ophthalmol* 1988; 106: 1190-5.
105 Fishman GA, Gilbert LD, Fiscella RG, Kimura AE, Jampol LM. Acetazolamide for treatment of chronic macular edema in retinitis pigmentosa. *Arch Ophthalmol* 1989; 107: 1445-52.
106 Chen JC, Fitzke FW, Bird AC. Long-term effect of acetazolamide in a patient with retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 1990; 31: 1914-8.
107 Berson EL, Rabin AR, Mehaffey L, III. Advances in night vision technology. A pocketscope for patients with retinitis pigmentosa. *Arch Ophthalmol* 1973; 90: 427-31.
108 Hartong DT, Jorritsma FF, Neve JJ, Melis-Dankers BJ, Kooijman AC. Improved mobility and independence of night-blind people using night-vision goggles. *Invest Ophthalmol Vis Sci* 2004; 45: 1725-31.
109 Mancil RM, Mancil GL, King E, Legault C, Munday J, Alfieri S, Nowakowski R, Blasch BB. Improving nighttime mobility in persons with night blindness caused by retinitis pigmentosa: A comparison of two low-vision mobility devices. *J Rehabil Res Dev* 2005; 42: 471-86.
110 Redmond TM, Yu S, Lee E, Bok D, Hamasaki D, Chen N, Goletz P, Ma JX, Crouch RK, Pfeifer K. Rpe65 is necessary for production of 11-cis-vitamin A in the retinal visual cycle. *Nat Genet* 1998; 20: 344-51.
111 Rohrer B, Goletz P, Znoiko S, Ablonczy Z, Ma JX, Redmond TM, Crouch RK. Correlation of regenerable opsin with rod ERG signal in Rpe65-/- mice during development and aging. *Invest Ophthalmol Vis Sci* 2003; 44: 310-5.
112 Acland GM, Aguirre GD, Ray J, Zhang Q, Aleman TS, Cideciyan AV, Pearce-Kelling SE, Anand V, Zeng Y, Maguire AM, Jacobson SG, Hauswirth WW, Bennett J. Gene therapy restores vision in a canine model of childhood blindness. *Nat Genet* 2001; 28: 92-5.
113 Dejneka NS, Surace EM, Aleman TS, Cideciyan AV, Lyubarsky A, Savchenko A, Redmond TM, Tang W, Wei Z, Rex TS, Glover E, Maguire AM, Pugh EN, Jr., Jacobson SG, Bennett J. In utero gene therapy rescues vision in a murine model of congenital blindness. *Mol Ther* 2004; 9: 182-8.

114 Jacobson SG, Aleman TS, Cideciyan AV, Sumaroka A, Schwartz SB, Windsor EA, Traboulsi EI, Heon E, Pittler SJ, Milam AH, Maguire AM, Palczewski K, Stone EM, Bennett J. Identifying photoreceptors in blind eyes caused by RPE65 mutations: Prerequisite for human gene therapy success. *Proc Natl Acad Sci U S A* 2005; 102: 6177-82.

115 Acland GM, Aguirre GD, Bennett J, Aleman TS, Cideciyan AV, Bennicelli J, Dejneka NS, Pearce-Kelling SE, Maguire AM, Palczewski K, Hauswirth WW, Jacobson SG. Long-Term Restoration of Rod and Cone Vision by Single Dose rAAV-Mediated Gene Transfer to the Retina in a Canine Model of Childhood Blindness. *Mol Ther* 2005; 12: 1072-82.

116 Bennett J, Tanabe T, Sun D, Zeng Y, Kjeldbye H, Gouras P, Maguire AM. Photoreceptor cell rescue in retinal degeneration (rd) mice by in vivo gene therapy. *Nat Med* 1996; 2: 649-54.

117 Jomary C, Vincent KA, Grist J, Neal MJ, Jones SE. Rescue of photoreceptor function by AAV-mediated gene transfer in a mouse model of inherited retinal degeneration. *Gene Ther* 1997; 4: 683-90.

118 Kumar-Singh R, Farber DB. Encapsidated adenovirus mini-chromosome-mediated delivery of genes to the retina: application to the rescue of photoreceptor degeneration. *Hum Mol Genet* 1998; 7: 1893-900.

119 Takahashi M, Miyoshi H, Verma IM, Gage FH. Rescue from photoreceptor degeneration in the rd mouse by human immunodeficiency virus vector-mediated gene transfer. *J Virol* 1999; 73: 7812-6.

120 Ali RR, Sarra GM, Stephens C, Alwis MD, Bainbridge JW, Munro PM, Fauser S, Reichel MB, Kinnon C, Hunt DM, Bhattacharya SS, Thrasher AJ. Restoration of photoreceptor ultrastructure and function in retinal degeneration slow mice by gene therapy. *Nat Genet* 2000; 25: 306-10.

121 Vollrath D, Feng W, Duncan JL, Yasumura D, D'Cruz PM, Chappelow A, Matthes MT, Kay MA, LaVail MM. Correction of the retinal dystrophy phenotype of the RCS rat by viral gene transfer of Mertk. *Proc Natl Acad Sci U S A* 2001; 98: 12584-9.

122 Farrar GJ, Kenna PF, Humphries P. On the genetics of retinitis pigmentosa and on mutation-independent approaches to therapeutic intervention. *EMBO J* 2002; 21: 857-64.

123 O'Neill B, Millington-Ward S, O'reilly M, Tuohy G, Kiang AS, Kenna PF, Humphries P, Farrar GJ. Ribozyme-based therapeutic approaches for autosomal dominant retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 2000; 41: 2863-9.

124 Cashman SM, Binkley EA, Kumar-Singh R. Towards mutation-independent silencing of genes involved in retinal degeneration by RNA interference. *Gene Ther* 2005; 12: 1223-8.

125 Lewin AS, Drenser KA, Hauswirth WW, Nishikawa S, Yasumura D, Flannery JG, LaVail MM. Ribozyme rescue of photoreceptor cells in a transgenic rat model of autosomal dominant retinitis pigmentosa. *Nat Med* 1998; 4: 967-71.

126 LaVail MM, Yasumura D, Matthes MT, Drenser KA, Flannery JG, Lewin AS, Hauswirth WW. Ribozyme rescue of photoreceptor cells in P23H transgenic rats: long-term survival and late-stage therapy. *Proc Natl Acad Sci U S A* 2000; 97: 11488-93.

127 Bennett J, Zeng Y, Bajwa R, Klatt L, Li Y, Maguire AM. Adenovirus-mediated delivery of rhodopsin-promoted bcl-2 results in a delay in photoreceptor cell death in the rd/rd mouse. *Gene Ther* 1998; 5: 1156-64.

128 Chen J, Flannery JG, LaVail MM, Steinberg RH, Xu J, Simon MI. bcl-2 overexpression reduces apoptotic photoreceptor cell death in three different retinal degenerations. *Proc Natl Acad Sci U S A* 1996; 93: 7042-7.

129 Nir I, Kedzierski W, Chen J, Travis GH. Expression of Bcl-2 protects against photoreceptor degeneration in retinal degeneration slow (rds) mice. *J Neurosci* 2000; 20: 2150-4.

130 Tsang SH, Chen J, Kjeldbye H, Li WS, Simon MI, Gouras P, Goff SP. Retarding photoreceptor degeneration in Pdegtm1/Pdegtml mice by an apoptosis suppressor gene. *Invest Ophthalmol Vis Sci* 1997; 38: 943-50.

131 Bode C, Wolfrum U. Caspase-3 inhibitor reduces apototic photoreceptor cell death during inherited retinal degeneration in tubby mice. *Mol Vis* 2003; 9: 144-50.

132 LaVail MM, Yasumura D, Matthes MT, Lau-Villacorta C, Unoki K, Sung CH, Steinberg RH. Protection of mouse photoreceptors by survival factors in retinal degenerations. *Invest Ophthalmol Vis Sci* 1998; 39: 592-602.
133 Leveillard T, Mohand-Said S, Lorentz O, Hicks D, Fintz AC, Clerin E, Simonutti M, Forster V, Cavusoglu N, Chalmel F, Dolle P, Poch O, Lambrou G, Sahel JA. Identification and characterization of rod-derived cone viability factor. *Nat Genet* 2004; 36: 755-9.
134 Otani A, Dorrell MI, Kinder K, Moreno SK, Nusinowitz S, Banin E, Heckenlively J, Friedlander M. Rescue of retinal degeneration by intravitreally injected adult bone marrow-derived lineage-negative hematopoietic stem cells. *J Clin Invest* 2004; 114: 765-74.
135 Dykens JA, Carroll AK, Wiley S, Covey DF, Cai ZY, Zhao L, Wen R. Photoreceptor preservation in the S334ter model of retinitis pigmentosa by a novel estradiol analog. *Biochem Pharmacol* 2004; 68: 1971-84.
136 Sieving PA, Caruso RC, Tao W, Coleman HR, Thompson DJ, Fullmer KR, Bush RA. Ciliary neurotrophic factor (CNTF) for human retinal degeneration: phase I trial of CNTF delivered by encapsulated cell intraocular implants. *Proc Natl Acad Sci U S A* 2006; 103: 3896-901.
137 Frasson M, Sahel JA, Fabre M, Simonutti M, Dreyfus H, Picaud S. Retinitis pigmentosa: rod photoreceptor rescue by a calcium-channel blocker in the rd mouse. *Nat Med* 1999; 5: 1183-7.
138 Pearce-Kelling SE, Aleman TS, Nickle A, Laties AM, Aguirre GD, Jacobson SG, Acland GM. Calcium channel blocker D-cis-diltiazem does not slow retinal degeneration in the PDE6B mutant rcd1 canine model of retinitis pigmentosa. *Mol Vis* 2001; 7: 42-7.
139 Bush RA, Kononen L, Machida S, Sieving PA. The effect of calcium channel blocker diltiazem on photoreceptor degeneration in the rhodopsin Pro213His rat. *Invest Ophthalmol Vis Sci* 2000; 41: 2697-701.
140 Pawlyk BS, Li T, Scimeca MS, Sandberg MA, Berson EL. Absence of photoreceptor rescue with D-cis-diltiazem in the rd mouse. *Invest Ophthalmol Vis Sci* 2002; 43: 1912-5.
141 Li LX, Turner JE. Transplantation of retinal pigment epithelial cells to immature and adult rat hosts: short- and long-term survival characteristics. *Exp Eye Res* 1988; 47: 771-85.
142 Lin N, Fan W, Sheedlo HJ, Aschenbrenner JE, Turner JE. Photoreceptor repair in response to RPE transplants in RCS rats: outer segment regeneration. *Curr Eye Res* 1996; 15: 1069-77.
143 Little CW, Castillo B, DiLoreto DA, Cox C, Wyatt J, del Cerro C, del Cerro M. Transplantation of human fetal retinal pigment epithelium rescues photoreceptor cells from degeneration in the Royal College of Surgeons rat retina. *Invest Ophthalmol Vis Sci* 1996; 37: 204-11.
144 Whiteley SJ, Litchfield TM, Coffey PJ, Lund RD. Improvement of the pupillary light reflex of Royal College of Surgeons rats following RPE cell grafts. *Exp Neurol* 1996; 140: 100-4.
145 Woch G, Aramant RB, Seiler MJ, Sagdullaev BT, McCall MA. Retinal transplants restore visually evoked responses in rats with photoreceptor degeneration. *Invest Ophthalmol Vis Sci* 2001; 42: 1669-76.
146 Berger AS, Tezel TH, Del Priore LV, Kaplan HJ. Photoreceptor transplantation in retinitis pigmentosa: short-term follow-up. *Ophthalmology* 2003; 110: 383-91.
147 Kim J, Wu HH, Lander AD, Lyons KM, Matzuk MM, Calof AL. GDF11 controls the timing of progenitor cell competence in developing retina. *Science* 2005; 308: 1927-30.
148 Sun G, Asami M, Ohta H, Kosaka J, Kosaka M. Retinal stem/progenitor properties of iris pigment epithelial cells. *Dev Biol* 2006; 289: 243-52.
149 Chiou SH, Kao CL, Peng CH, Chen SJ, Tarng YW, Ku HH, Chen YC, Shyr YM, Liu RS, Hsu CJ, Yang DM, Hsu WM, Kuo CD, Lee CH. A novel in vitro retinal differentiation model by co-culturing adult human bone marrow stem cells with retinal pigmented epithelium cells. *Biochem Biophys Res Commun* 2005; 326: 578-85.
150 Angenieux B, Schorderet DF, Arsenijevic Y. Epidermal growth factor is a neuronal differentiation factor for retinal stem cells in vitro. *Stem Cells* 2006; 24: 696-706.

151 Meyer JS, Katz ML, Maruniak JA, Kirk MD. Embryonic stem cell-derived neural progenitors incorporate into degenerating retina and enhance survival of host photoreceptors. *Stem Cells* 2006; 24: 274-83.
152 Banin E, Obolensky A, Idelson M, Hemo I, Reinhardtz E, Pikarsky E, Ben Hur T, Reubinoff B. Retinal incorporation and differentiation of neural precursors derived from human embryonic stem cells. *Stem Cells* 2006; 24: 246-57.
153 Seiler MJ, Aramant RB. Transplantation of neuroblastic progenitor cells as a sheet preserves and restores retinal function. *Semin Ophthalmol* 2005; 20: 31-42.
154 Coles BL, Angenieux B, Inoue T, Rio-Tsonis K, Spence JR, McInnes RR, Arsenijevic Y, van der KD. Facile isolation and the characterization of human retinal stem cells. *Proc Natl Acad Sci U S A* 2004; 101: 15772-7.
155 Radtke ND, Aramant RB, Seiler MJ, Petry HM, Pidwell D. Vision change after sheet transplant of fetal retina with retinal pigment epithelium to a patient with retinitis pigmentosa. *Arch Ophthalmol* 2004; 122: 1159-65.
156 Chow AY, Chow VY, Packo KH, Pollack JS, Peyman GA, Schuchard R. The artificial silicon retina microchip for the treatment of vision loss from retinitis pigmentosa. *Arch Ophthalmol* 2004; 122: 460-9.
157 Pardue MT, Phillips MJ, Yin H, Fernandes A, Cheng Y, Chow AY, Ball SL. Possible sources of neuroprotection following subretinal silicon chip implantation in RCS rats. *J Neural Eng* 2005; 2: S39-S47.
158 Jensen RJ, Ziv OR, Rizzo JF, III. Thresholds for activation of rabbit retinal ganglion cells with relatively large, extracellular microelectrodes. *Invest Ophthalmol Vis Sci* 2005; 46: 1486-96.
159 Brelen ME, Duret F, Gerard B, Delbeke J, Veraart C. Creating a meaningful visual perception in blind volunteers by optic nerve stimulation. *J Neural Eng* 2005; 2: S22-S28.
160 Fang X, Sakaguchi H, Fujikado T, Osanai M, Kanda H, Ikuno Y, Kamei M, Ohji M, Gan D, Choi J, Yagi T, Tano Y. Direct stimulation of optic nerve by electrodes implanted in optic disc of rabbit eyes. *Graefes Arch Clin Exp Ophthalmol* 2005; 243: 49-56.
161 Schmidt EM, Bak MJ, Hambrecht FT, Kufta CV, O'Rourke DK, Vallabhanath P. Feasibility of a visual prosthesis for the blind based on intracortical microstimulation of the visual cortex. *Brain* 1996; 119 (Pt 2): 507-22.
162 Lee HW, Hong SB, Seo DW, Tae WS, Hong SC. Mapping of functional organization in human visual cortex: electrical cortical stimulation. *Neurology* 2000; 54: 849-54.
163 Gekeler F, Szurman P, Grisanti S, Weiler U, Claus R, Greiner TO, Volker M, Kohler K, Zrenner E, Bartz-Schmidt KU. Compound subretinal prostheses with extra-ocular parts designed for human trials: successful long-term implantation in pigs. *Graefes Arch Clin Exp Ophthalmol* 2006; *(in press).*
164 Rizzo JF, III, Wyatt J, Loewenstein J, Kelly S, Shire D. Perceptual efficacy of electrical stimulation of human retina with a microelectrode array during short-term surgical trials. *Invest Ophthalmol Vis Sci* 2003; 44: 5362-9.
165 Mahadevappa M, Weiland JD, Yanai D, Fine I, Greenberg RJ, Humayun MS. Perceptual thresholds and electrode impedance in three retinal prosthesis subjects. *IEEE Trans Neural Syst Rehabil Eng* 2005; 13: 201-6.
166 Kamei M, Fujikado T, Kanda H, Morimoto T, Nakauchi K, Sakaguchi H, Ikuno Y, Ozawa M, Kusaka S, Tano Y. Suprachoroidal-Transretinal Stimulation (STS) Artificial Vision System for Patients with Retinitis Pigmentosa. *Invest Ophthalmol Vis Sci* 2006; 47: ARVO E-abstract 1537.
167 Zrenner E, Besch D, Bartz-Schmidt KU, Gekeler F, Gabel VP, Kuttenkeuler C, Sachs H, Sailer H, Wilhelm B, Wilke R. Subretinal Chronic Multi-Electrode Arrays Implanted in Blind Patients. *Invest Ophthalmol Vis Sci* 2006; 47: ARVO E-abstract 1538.

168 Klevering BJ, Yzer S, Rohrschneider K, Zonneveld M, Allikmets R, van den Born LI, Maugeri A, Hoyng CB, Cremers FP. Microarray-based mutation analysis of the ABCA4 (ABCR) gene in autosomal recessive cone-rod dystrophy and retinitis pigmentosa. *Eur J Hum Genet* 2004; 12: 1024-32.

169 Tuson M, Marfany G, Gonzalez-Duarte R. Mutation of CERKL, a novel human ceramide kinase gene, causes autosomal recessive retinitis pigmentosa (RP26). *Am J Hum Genet* 2004; 74: 128-38.

170 Dryja TP, Finn JT, Peng YW, McGee TL, Berson EL, Yau KW. Mutations in the gene encoding the alpha subunit of the rod cGMP-gated channel in autosomal recessive retinitis pigmentosa. *Proc Natl Acad Sci U S A* 1995; 92: 10177-81.

171 Bareil C, Hamel CP, Delague V, Arnaud B, Demaille J, Claustres M. Segregation of a mutation in CNGB1 encoding the beta-subunit of the rod cGMP-gated channel in a family with autosomal recessive retinitis pigmentosa. *Hum Genet* 2001; 108: 328-34.

172 den Hollander AI, Davis J, van der Velde-Visser SD, Zonneveld MN, Pierrottet CO, Koenekoop RK, Kellner U, van den Born LI, Heckenlively JR, Hoyng CB, Handford PA, Roepman R, Cremers FP. CRB1 mutation spectrum in inherited retinal dystrophies. *Hum Mutat* 2004; 24: 355-69.

173 Thompson DA, Li Y, McHenry CL, Carlson TJ, Ding X, Sieving PA, Apfelstedt-Sylla E, Gal A. Mutations in the gene encoding lecithin retinol acyltransferase are associated with early-onset severe retinal dystrophy. *Nat Genet* 2001; 28: 123-4.

174 Gal A, Li Y, Thompson DA, Weir J, Orth U, Jacobson SG, Apfelstedt-Sylla E, Vollrath D. Mutations in MERTK, the human orthologue of the RCS rat retinal dystrophy gene, cause retinitis pigmentosa. *Nat Genet* 2000; 26: 270-1.

175 Sharon D, Sandberg MA, Caruso RC, Berson EL, Dryja TP. Shared mutations in NR2E3 in enhanced S-cone syndrome, Goldmann-Favre syndrome, and many cases of clumped pigmentary retinal degeneration. *Arch Ophthalmol* 2003; 121: 1316-23.

176 To KW, Adamian M, Jakobiec FA, Berson EL. Clinical and histopathologic findings in clumped pigmentary retinal degeneration. *Arch Ophthalmol* 1996; 114: 950-5.

177 Nishiguchi KM, Friedman JS, Sandberg MA, Swaroop A, Berson EL, Dryja TP. Recessive NRL mutations in patients with clumped pigmentary retinal degeneration and relative preservation of blue cone function. *Proc Natl Acad Sci U S A* 2004; 101: 17819-24.

178 Dryja TP, Rucinski DE, Chen SH, Berson EL. Frequency of mutations in the gene encoding the alpha subunit of rod cGMP-phosphodiesterase in autosomal recessive retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 1999; 40: 1859-65.

179 Bayes M, Giordano M, Balcells S, Grinberg D, Vilageliu L, Martinez I, Ayuso C, Benitez J, Ramos-Arroyo MA, Chivelet P, . Homozygous tandem duplication within the gene encoding the beta-subunit of rod phosphodiesterase as a cause for autosomal recessive retinitis pigmentosa. *Hum Mutat* 1995; 5: 228-34.

180 McLaughlin ME, Ehrhart TL, Berson EL, Dryja TP. Mutation spectrum of the gene encoding the beta subunit of rod phosphodiesterase among patients with autosomal recessive retinitis pigmentosa. *Proc Natl Acad Sci U S A* 1995; 92: 3249-53.

181 Morimura H, Saindelle-Ribeaudeau F, Berson EL, Dryja TP. Mutations in RGR, encoding a light-sensitive opsin homologue, in patients with retinitis pigmentosa. *Nat Genet* 1999; 23: 393-4.

182 Rosenfeld PJ, Cowley GS, McGee TL, Sandberg MA, Berson EL, Dryja TP. A null mutation in the rhodopsin gene causes rod photoreceptor dysfunction and autosomal recessive retinitis pigmentosa. *Nat Genet* 1992; 1: 209-13.

183 Kumaramanickavel G, Maw M, Denton MJ, John S, Srikumari CR, Orth U, Oehlmann R, Gal A. Missense rhodopsin mutation in a family with recessive RP. *Nat Genet* 1994; 8: 10-1.

184 Burstedt MS, Sandgren O, Holmgren G, Forsman-Semb K. Bothnia dystrophy caused by mutations in the cellular retinaldehyde-binding protein gene (RLBP1) on chromosome 15q26. *Invest Ophthalmol Vis Sci* 1999; 40: 995-1000.
185 Morimura H, Berson EL, Dryja TP. Recessive mutations in the RLBP1 gene encoding cellular retinaldehyde-binding protein in a form of retinitis punctata albescens. *Invest Ophthalmol Vis Sci* 1999; 40: 1000-4.
186 Khaliq S, Abid A, Ismail M, Hameed A, Mohyuddin A, Lall P, Aziz A, Anwar K, Mehdi SQ. Novel association of RP1 gene mutations with autosomal recessive retinitis pigmentosa. *J Med Genet* 2005; 42: 436-8.
187 Riazuddin SA, Zulfiqar F, Zhang Q, Sergeev YV, Qazi ZA, Husnain T, Caruso R, Riazuddin S, Sieving PA, Hejtmancik JF. Autosomal recessive retinitis pigmentosa is associated with mutations in RP1 in three consanguineous Pakistani families. *Invest Ophthalmol Vis Sci* 2005; 46: 2264-70.
188 Morimura H, Fishman GA, Grover SA, Fulton AB, Berson EL, Dryja TP. Mutations in the RPE65 gene in patients with autosomal recessive retinitis pigmentosa or leber congenital amaurosis. *Proc Natl Acad Sci U S A* 1998; 95: 3088-93.
189 Nakazawa M, Wada Y, Tamai M. Arrestin gene mutations in autosomal recessive retinitis pigmentosa. *Arch Ophthalmol* 1998; 116: 498-501.
190 Hagstrom SA, North MA, Nishina PL, Berson EL, Dryja TP. Recessive mutations in the gene encoding the tubby-like protein TULP1 in patients with retinitis pigmentosa. *Nat Genet* 1998; 18: 174-6.
191 Seyedahmadi BJ, Rivolta C, Keene JA, Berson EL, Dryja TP. Comprehensive screening of the USH2A gene in Usher syndrome type II and non-syndromic recessive retinitis pigmentosa. *Exp Eye Res* 2004; 79: 167-73.
192 Ouyang XM, Hejtmancik JF, Jacobson SG, Li AR, Du LL, Angeli S, Kaiser M, Balkany T, Liu XZ. Mutational spectrum in Usher syndrome type II. *Clin Genet* 2004; 65: 288-93.
193 Katsanis N. The oligogenic properties of Bardet-Biedl syndrome. *Hum Mol Genet* 2004; 13 Spec No 1: R65-R71.
194 Ouyang XM, Yan D, Du LL, Hejtmancik JF, Jacobson SG, Nance WE, Li AR, Angeli S, Kaiser M, Newton V, Brown SD, Balkany T, Liu XZ. Characterization of Usher syndrome type I gene mutations in an Usher syndrome patient population. *Hum Genet* 2005; 116: 292-9.
195 Aller E, Jaijo T, Oltra S, Alio J, Galan F, Najera C, Beneyto M, Millan JM. Mutation screening of USH3 gene (clarin-1) in Spanish patients with Usher syndrome: low prevalence and phenotypic variability. *Clin Genet* 2004; 66: 525-9.
196 Dryja TP, McEvoy JA, McGee TL, Berson EL. Novel rhodopsin mutations Gly114Val and Gln184Pro in dominant retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 2000; 41: 3124-7.
197 Bunge S, Wedemann H, David D, Terwilliger DJ, van den Born LI, Aulehla-Scholz C, Samanns C, Horn M, Ott J, Schwinger E, . Molecular analysis and genetic mapping of the rhodopsin gene in families with autosomal dominant retinitis pigmentosa. *Genomics* 1993; 17: 230-3.
198 Sohocki MM, Daiger SP, Bowne SJ, Rodriquez JA, Northrup H, Heckenlively JR, Birch DG, Mintz-Hittner H, Ruiz RS, Lewis RA, Saperstein DA, Sullivan LS. Prevalence of mutations causing retinitis pigmentosa and other inherited retinopathies. *Hum Mutat* 2001; 17: 42-51.
199 Berson EL, Grimsby JL, Adams SM, McGee TL, Sweklo E, Pierce EA, Sandberg MA, Dryja TP. Clinical features and mutations in patients with dominant retinitis pigmentosa-1 (RP1). *Invest Ophthalmol Vis Sci* 2001; 42: 2217-24.
200 Dryja TP, Hahn LB, Kajiwara K, Berson EL. Dominant and digenic mutations in the peripherin/RDS and ROM1 genes in retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 1997; 38: 1972-82.
201 Wada Y, Sandberg MA, McGee TL, Stillberger MA, Berson EL, Dryja TP. Screen of the IMPDH1 gene among patients with dominant retinitis pigmentosa and clinical features associated with the most common mutation, Asp226Asn. *Invest Ophthalmol Vis Sci* 2005; 46: 1735-41.

202 Bowne SJ, Sullivan LS, Mortimer SE, Hedstrom L, Zhu J, Spellicy CJ, Gire AI, Hughbanks-Wheaton D, Birch DG, Lewis RA, Heckenlively JR, Daiger SP. Spectrum and Frequency of Mutations in IMPDH1 Associated with Autosomal Dominant Retinitis Pigmentosa and Leber Congenital Amaurosis. *Invest Ophthalmol Vis Sci* 2006; 47: 34-42.

203 DeAngelis MM, Grimsby JL, Sandberg MA, Berson EL, Dryja TP. Novel mutations in the NRL gene and associated clinical findings in patients with dominant retinitis pigmentosa. *Arch Ophthalmol* 2002; 120: 369-75.

204 Rivolta C, Peck NE, Fulton AB, Fishman GA, Berson EL, Dryja TP. Novel frameshift mutations in CRX associated with Leber congenital amaurosis. *Hum Mutat* 2001; 18: 550-1.

205 Rebello G, Ramesar R, Vorster A, Roberts L, Ehrenreich L, Oppon E, Gama D, Bardien S, Greenberg J, Bonapace G, Waheed A, Shah GN, Sly WS. Apoptosis-inducing signal sequence mutation in carbonic anhydrase IV identified in patients with the RP17 form of retinitis pigmentosa. *Proc Natl Acad Sci U S A* 2004; 101: 6617-22.

206 Wada Y, Abe T, Takeshita T, Sato H, Yanashima K, Tamai M. Mutation of human retinal fascin gene (FSCN2) causes autosomal dominant retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 2001; 42: 2395-400.

207 Sato M, Nakazawa M, Usui T, Tanimoto N, Abe H, Ohguro H. Mutations in the gene coding for guanylate cyclase-activating protein 2 (GUCA1B gene) in patients with autosomal dominant retinal dystrophies. *Graefes Arch Clin Exp Ophthalmol* 2005; 243: 235-42.

208 Keen TJ, Hims MM, McKie AB, Moore AT, Doran RM, Mackey DA, Mansfield DC, Mueller RF, Bhattacharya SS, Bird AC, Markham AF, Inglehearn CF. Mutations in a protein target of the Pim-1 kinase associated with the RP9 form of autosomal dominant retinitis pigmentosa. *Eur J Hum Genet* 2002; 10: 245-9.

209 Abid A, Ismail M, Mehdi SQ, Khaliq S. Identification of novel mutations in SEMA4A gene associated with retinal degenerative diseases. *J Med Genet* 2006; 43: 378-81.

210 Sharon D, Sandberg MA, Rabe VW, Stillberger M, Dryja TP, Berson EL. RP2 and RPGR mutations and clinical correlations in patients with X-linked retinitis pigmentosa. *Am J Hum Genet* 2003; 73: 1131-46.

211 Bader I, Brandau O, Achatz H, Apfelstedt-Sylla E, Hergersberg M, Lorenz B, Wissinger B, Wittwer B, Rudolph G, Meindl A, Meitinger T. X-linked retinitis pigmentosa: RPGR mutations in most families with definite X linkage and clustering of mutations in a short sequence stretch of exon ORF15. *Invest Ophthalmol Vis Sci* 2003; 44: 1458-63.

212 Breuer DK, Swaroop A., Musarella M. Verification and fine mapping of the X-linked retinitis pigmentosa locus RP6. Invest. Ophthalmol. Vis. Sci. 41:S191 (2000). *Invest Ophthalmol Vis Sci* 2000; 41: S191.

213 Hardcastle AJ, Thiselton DL, Zito I, Ebenezer N, Mah TS, Gorin MB, Bhattacharya SS. Evidence for a new locus for X-linked retinitis pigmentosa (RP23). *Invest Ophthalmol Vis Sci* 2000; 41: 2080-6.

214 Gieser L, Fujita R, Goring HH, Ott J, Hoffman DR, Cideciyan AV, Birch DG, Jacobson SG, Swaroop A. A novel locus (RP24) for X-linked retinitis pigmentosa maps to Xq26-27. *Am J Hum Genet* 1998; 63: 1439-47.

215 Hargrave PA. Rhodopsin structure, function, and topography the Friedenwald lecture. *Invest Ophthalmol Vis Sci* 2001; 42: 3-9.

216 Koutalos Y, Nakatani K, Yau KW. The cGMP-phosphodiesterase and its contribution to sensitivity regulation in retinal rods. *J Gen Physiol* 1995; 106: 891-921.

217 Fung BK, Young JH, Yamane HK, Griswold-Prenner I. Subunit stoichiometry of retinal rod cGMP phosphodiesterase. *Biochemistry* 1990; 29: 2657-64.

218 Dhallan RS, Macke JP, Eddy RL, Shows TB, Reed RR, Yau KW, Nathans J. Human rod photoreceptor cGMP-gated channel: amino acid sequence, gene structure, and functional expression. *J Neurosci* 1992; 12: 3248-56.

219 Korschen HG, Beyermann M, Muller F, Heck M, Vantler M, Koch KW, Kellner R, Wolfrum U, Bode C, Hofmann KP, Kaupp UB. Interaction of glutamic-acid-rich proteins with the cGMP signalling pathway in rod photoreceptors. *Nature* 1999; 400: 761-6.
220 Poetsch A, Molday LL, Molday RS. The cGMP-gated channel and related glutamic acid-rich proteins interact with peripherin-2 at the rim region of rod photoreceptor disc membranes. *J Biol Chem* 2001; 276: 48009-16.
221 Batra-Safferling R, Abarca-Heidemann K, Korschen HG, Tziatzios C, Stoldt M, Budyak I, Willbold D, Schwalbe H, Klein-Seetharaman J, Kaupp UB. Glutamic Acid-rich Proteins of Rod Photoreceptors Are Natively Unfolded. *J Biol Chem* 2006; 281: 1449-60.
222 Palczewski K, McDowell JH, Jakes S, Ingebritsen TS, Hargrave PA. Regulation of rhodopsin dephosphorylation by arrestin. *J Biol Chem* 1989; 264: 15770-3.
223 Weng J, Mata NL, Azarian SM, Tzekov RT, Birch DG, Travis GH. Insights into the function of Rim protein in photoreceptors and etiology of Stargardt's disease from the phenotype in abcr knockout mice. *Cell* 1999; 98: 13-23.
224 Sun H, Nathans J. Mechanistic studies of ABCR, the ABC transporter in photoreceptor outer segments responsible for autosomal recessive Stargardt disease. *J Bioenerg Biomembr* 2001; 33: 523-30.
225 Saari JC, Nawrot M, Kennedy BN, Garwin GG, Hurley JB, Huang J, Possin DE, Crabb JW. Visual cycle impairment in cellular retinaldehyde binding protein (CRALBP) knockout mice results in delayed dark adaptation. *Neuron* 2001; 29: 739-48.
226 Xue L, Gollapalli DR, Maiti P, Jahng WJ, Rando RR. A palmitoylation switch mechanism in the regulation of the visual cycle. *Cell* 2004; 117: 761-71.
227 Moiseyev G, Chen Y, Takahashi Y, Wu BX, Ma JX. RPE65 is the isomerohydrolase in the retinoid visual cycle. *Proc Natl Acad Sci U S A* 2005; 102: 12413-8.
228 Chen P, Hao W, Rife L, Wang XP, Shen D, Chen J, Ogden T, Van Boemel GB, Wu L, Yang M, Fong HK. A photic visual cycle of rhodopsin regeneration is dependent on Rgr. *Nat Genet* 2001; 28: 256-60.
229 Travis GH, Sutcliffe JG, Bok D. The retinal degeneration slow (rds) gene product is a photoreceptor disc membrane-associated glycoprotein. *Neuron* 1991; 6: 61-70.
230 Connell G, Bascom R, Molday L, Reid D, McInnes RR, Molday RS. Photoreceptor peripherin is the normal product of the gene responsible for retinal degeneration in the rds mouse. *Proc Natl Acad Sci U S A* 1991; 88: 723-6.
231 Clarke G, Goldberg AF, Vidgen D, Collins L, Ploder L, Schwarz L, Molday LL, Rossant J, Szel A, Molday RS, Birch DG, McInnes RR. Rom-1 is required for rod photoreceptor viability and the regulation of disk morphogenesis. *Nat Genet* 2000; 25: 67-73.
232 Saishin Y, Ishikawa R, Ugawa S, Guo W, Ueda T, Morimura H, Kohama K, Shimizu H, Tano Y, Shimada S. Retinal fascin: functional nature, subcellular distribution, and chromosomal localization. *Invest Ophthalmol Vis Sci* 2000; 41: 2087-95.
233 Tubb BE, Bardien-Kruger S, Kashork CD, Shaffer LG, Ramagli LS, Xu J, Siciliano MJ, Bryan J. Characterization of human retinal fascin gene (FSCN2) at 17q25: close physical linkage of fascin and cytoplasmic actin genes. *Genomics* 2000; 65: 146-56.
234 Xi Q, Pauer GJ, Marmorstein AD, Crabb JW, Hagstrom SA. Tubby-like protein 1 (TULP1) interacts with F-actin in photoreceptor cells. *Invest Ophthalmol Vis Sci* 2005; 46: 4754-61.
235 Pellikka M, Tanentzapf G, Pinto M, Smith C, McGlade CJ, Ready DF, Tepass U. Crumbs, the Drosophila homologue of human CRB1/RP12, is essential for photoreceptor morphogenesis. *Nature* 2002; 416: 143-9.
236 Liu Q, Zuo J, Pierce EA. The retinitis pigmentosa 1 protein is a photoreceptor microtubule-associated protein. *J Neurosci* 2004; 24: 6427-36.
237 Rice DS, Huang W, Jones HA, Hansen G, Ye GL, Xu N, Wilson EA, Troughton K, Vaddi K, Newton RC, Zambrowicz BP, Sands AT. Severe retinal degeneration associated with disruption of semaphorin 4A. *Invest Ophthalmol Vis Sci* 2004; 45: 2767-77.

238 Boeda B, El Amraoui A, Bahloul A, Goodyear R, Daviet L, Blanchard S, Perfettini I, Fath KR, Shorte S, Reiners J, Houdusse A, Legrain P, Wolfrum U, Richardson G, Petit C. Myosin VIIa, harmonin and cadherin 23, three Usher I gene products that cooperate to shape the sensory hair cell bundle. *EMBO J* 2002; 21: 6689-99.

239 Siemens J, Kazmierczak P, Reynolds A, Sticker M, Littlewood-Evans A, Muller U. The Usher syndrome proteins cadherin 23 and harmonin form a complex by means of PDZ-domain interactions. *Proc Natl Acad Sci U S A* 2002; 99: 14946-51.

240 Ahmed ZM, Riazuddin S, Ahmad J, Bernstein SL, Guo Y, Sabar MF, Sieving P, Riazuddin S, Griffith AJ, Friedman TB, Belyantseva IA, Wilcox ER. PCDH15 is expressed in the neurosensory epithelium of the eye and ear and mutant alleles are responsible for both USH1F and DFNB23. *Hum Mol Genet* 2003; 12: 3215-23.

241 Reiners J, Van Wijk E, Marker T, Zimmermann U, Jurgens K, Te BH, Overlack N, Roepman R, Knipper M, Kremer H, Wolfrum U. Scaffold protein harmonin (USH1C) provides molecular links between Usher syndrome type 1 and type 2. *Hum Mol Genet* 2005; 14: 3933-43.

242 Adato A, Vreugde S, Joensuu T, Avidan N, Hamalainen R, Belenkiy O, Olender T, Bonne-Tamir B, Ben Asher E, Espinos C, Millan JM, Lehesjoki AE, Flannery JG, Avraham KB, Pietrokovski S, Sankila EM, Beckmann JS, Lancet D. USH3A transcripts encode clarin-1, a four-transmembrane-domain protein with a possible role in sensory synapses. *Eur J Hum Genet* 2002; 10: 339-50.

243 Chapple JP, Grayson C, Hardcastle AJ, Bailey TA, Matter K, Adamson P, Graham CH, Willison KR, Cheetham ME. Organization on the plasma membrane of the retinitis pigmentosa protein RP2: investigation of association with detergent-resistant membranes and polarized sorting. *Biochem J* 2003; 372: 427-33.

244 Zhou Z, Licklider LJ, Gygi SP, Reed R. Comprehensive proteomic analysis of the human spliceosome. *Nature* 2002; 419: 182-5.

245 Umen JG, Guthrie C. Prp16p, Slu7p, and Prp8p interact with the 3' splice site in two distinct stages during the second catalytic step of pre-mRNA splicing. *RNA* 1995; 1: 584-97.

246 Lauber J, Plessel G, Prehn S, Will CL, Fabrizio P, Groning K, Lane WS, Luhrmann R. The human U4/U6 snRNP contains 60 and 90kD proteins that are structurally homologous to the yeast splicing factors Prp4p and Prp3p. *RNA* 1997; 3: 926-41.

247 Wang A, Forman-Kay J, Luo Y, Luo M, Chow YH, Plumb J, Friesen JD, Tsui LC, Heng HH, Woolford JL, Jr., Hu J. Identification and characterization of human genes encoding Hprp3p and Hprp4p, interacting components of the spliceosome. *Hum Mol Genet* 1997; 6: 2117-26.

248 Maita H, Kitaura H, Keen TJ, Inglehearn CF, Ariga H, Iguchi-Ariga SM. PAP-1, the mutated gene underlying the RP9 form of dominant retinitis pigmentosa, is a splicing factor. *Exp Cell Res* 2004; 300: 283-96.

249 Gibbs D, Azarian SM, Lillo C, Kitamoto J, Klomp AE, Steel KP, Libby RT, Williams DS. Role of myosin VIIa and Rab27a in the motility and localization of RPE melanosomes. *J Cell Sci* 2004; 117: 6473-83.

250 Adato A, Michel V, Kikkawa Y, Reiners J, Alagramam KN, Weil D, Yonekawa H, Wolfrum U, El Amraoui A, Petit C. Interactions in the network of Usher syndrome type 1 proteins. *Hum Mol Genet* 2005; 14: 347-56.

251 Yen HJ, Tayeh MK, Mullins RF, Stone EM, Sheffield VC, Slusarski DC. Bardet-Biedl syndrome genes are important in retrograde intracellular trafficking and Kupffer's vesicle cilia function. *Hum Mol Genet* 2006; 15: 667-77.

252 Nishimura DY, Fath M, Mullins RF, Searby C, Andrews M, Davis R, Andorf JL, Mykytyn K, Swiderski RE, Yang B, Carmi R, Stone EM, Sheffield VC. Bbs2-null mice have neurosensory deficits, a defect in social dominance, and retinopathy associated with mislocalization of rhodopsin. *Proc Natl Acad Sci U S A* 2004; 101: 16588-93.

253 Mykytyn K, Mullins RF, Andrews M, Chiang AP, Swiderski RE, Yang B, Braun T, Casavant T, Stone EM, Sheffield VC. Bardet-Biedl syndrome type 4 (BBS4)-null mice implicate Bbs4 in flagella formation but not global cilia assembly. *Proc Natl Acad Sci U S A* 2004; 101: 8664-9.

254 Kim JC, Badano JL, Sibold S, Esmail MA, Hill J, Hoskins BE, Leitch CC, Venner K, Ansley SJ, Ross AJ, Leroux MR, Katsanis N, Beales PL. The Bardet-Biedl protein BBS4 targets cargo to the pericentriolar region and is required for microtubule anchoring and cell cycle progression. *Nat Genet* 2004; 36: 462-70.

255 Li JB, Gerdes JM, Haycraft CJ, Fan Y, Teslovich TM, May-Simera H, Li H, Blacque OE, Li L, Leitch CC, Lewis RA, Green JS, Parfrey PS, Leroux MR, Davidson WS, Beales PL, Guay-Woodford LM, Yoder BK, Stormo GD, Katsanis N, Dutcher SK. Comparative genomics identifies a flagellar and basal body proteome that includes the BBS5 human disease gene. *Cell* 2004; 117: 541-52.

256 Fath MA, Mullins RF, Searby C, Nishimura DY, Wei J, Rahmouni K, Davis RE, Tayeh MK, Andrews M, Yang B, Sigmund CD, Stone EM, Sheffield VC. Mkks-null mice have a phenotype resembling Bardet-Biedl syndrome. *Hum Mol Genet* 2005; 14: 1109-18.

257 Blacque OE, Reardon MJ, Li C, McCarthy J, Mahjoub MR, Ansley SJ, Badano JL, Mah AK, Beales PL, Davidson WS, Johnsen RC, Audeh M, Plasterk RH, Baillie DL, Katsanis N, Quarmby LM, Wicks SR, Leroux MR. Loss of C. elegans BBS-7 and BBS-8 protein function results in cilia defects and compromised intraflagellar transport. *Genes Dev* 2004; 18: 1630-42.

258 Ansley SJ, Badano JL, Blacque OE, Hill J, Hoskins BE, Leitch CC, Kim JC, Ross AJ, Eichers ER, Teslovich TM, Mah AK, Johnsen RC, Cavender JC, Lewis RA, Leroux MR, Beales PL, Katsanis N. Basal body dysfunction is a likely cause of pleiotropic Bardet-Biedl syndrome. *Nature* 2003; 425: 628-33.

259 Hong DH, Pawlyk B, Sokolov M, Strissel KJ, Yang J, Tulloch B, Wright AF, Arshavsky VY, Li T. RPGR isoforms in photoreceptor connecting cilia and the transitional zone of motile cilia. *Invest Ophthalmol Vis Sci* 2003; 44: 2413-21.

260 Khanna H, Hurd TW, Lillo C, Shu X, Parapuram SK, He S, Akimoto M, Wright AF, Margolis B, Williams DS, Swaroop A. RPGR-ORF15, which is mutated in retinitis pigmentosa, associates with SMC1, SMC3, and microtubule transport proteins. *J Biol Chem* 2005; 280: 33580-7.

261 Yang Z, Alvarez BV, Chakarova C, Jiang L, Karan G, Frederick JM, Zhao Y, Sauve Y, Li X, Zrenner E, Wissinger B, Hollander AI, Katz B, Baehr W, Cremers FP, Casey JR, Bhattacharya SS, Zhang K. Mutant carbonic anhydrase 4 impairs pH regulation and causes retinal photoreceptor degeneration. *Hum Mol Genet* 2005; 14: 255-65.

262 Vollrath D, Feng W, Duncan JL, Yasumura D, D'Cruz PM, Chappelow A, Matthes MT, Kay MA, LaVail MM. Correction of the retinal dystrophy phenotype of the RCS rat by viral gene transfer of Mertk. *Proc Natl Acad Sci U S A* 2001; 98: 12584-9.

263 Bornancin F, Mechtcheriakova D, Stora S, Graf C, Wlachos A, Devay P, Urtz N, Baumruker T, Billich A. Characterization of a ceramide kinase-like protein. *Biochim Biophys Acta* 2005; 1687: 31-43.

264 Bowne SJ, Sullivan LS, Blanton SH, Cepko CL, Blackshaw S, Birch DG, Hughbanks-Wheaton D, Heckenlively JR, Daiger SP. Mutations in the inosine monophosphate dehydrogenase 1 gene (IMPDH1) cause the RP10 form of autosomal dominant retinitis pigmentosa. *Hum Mol Genet* 2002; 11: 559-68.

Chapter 2

Insights from retinitis pigmentosa into the roles of isocitrate dehydrogenases in the Krebs cycle

D.T. Hartong,[1A, 3 §] M. Dange,[2§] T.L. McGee,[1A] E.L. Berson,[1B]
T.P. Dryja,[1A] and R.F. Colman[2]

[A]Ocular Molecular Genetics Institute, Boston, MA, USA
[B]The Berman-Gund Laboratory for the Study of Retinal Degenerations, Boston, MA, USA
[1]Harvard Medical School, Massachusetts Eye and Ear Infirmary, Boston, MA, USA
[2]Dept. of Chemistry and Biochemistry, University of Delaware, Newark, DE, USA
[3]Dept. of Ophthalmology, University Medical Center Groningen, Groningen, The Netherlands
[§]These authors contributed equally to the work

Nature Genetics 40, 1230 - 1234 (2008)

Abstract

Here we describe two families with retinitis pigmentosa, a hereditary neurodegeneration of rod and cone photoreceptors in the retina. Affected family members were homozygous for loss-of-function mutations in *IDH3B*, encoding the β-subunit of NAD-specific isocitrate dehydrogenase (NAD-IDH, or IDH3), which is believed to catalyze the oxidation of isocitrate to α-ketoglutarate in the citric acid cycle. Cells from affected individuals had a substantial reduction of NAD-IDH activity, with about a 300-fold increase in the K_m for NAD. NADP-specific isocitrate dehydrogenase (NADP-IDH, or IDH2), an enzyme that catalyzes the same reaction, was normal in affected individuals, and they had no health problems associated with the enzyme deficiency except for retinitis pigmentosa. These findings support the hypothesis that mitochondrial NADP-IDH, rather than NAD-IDH, serves as the main catalyst for this reaction in the citric acid cycle outside the retina, and that the retina has a particular requirement for NAD-IDH.

Mutations in at least 34 genes have been identified as causes of nonsyndromic retinitis pigmentosa, including dominant mutations in 15 genes, recessive mutations in 20 genes and X-linked mutations in 2 genes (data from RetNet; see Methods for URL). The identified mutations are estimated to account for about 60% of cases of retinitis pigmentosa[1]. In addition, 11 unidentified genes have been mapped to specific chromosomal regions. There likely are dozens of unmapped, unidentified genes with recessive mutations associated with retinitis pigmentosa, each accounting for at most a few percent of cases[1,2].

We searched for some of these unidentified genes using an approach that relies on the fact that recessive alleles are often deletions, frameshift mutations or nonsense mutations that result in a scarcity of the disease gene's transcript because the transcript is either not made or rapidly degraded through nonsense-mediated decay[3]. The principle underlying this method has been described[4,5,6], but to our knowledge, the method has not been used to identify a human gene associated with a hereditary disease. Using microarray techniques that simultaneously assay mRNA levels from tens of thousands of transcripts in affected individuals, we searched for genes with absent or very low expression that may result from two allelic disease-causing mutations. It would have been ideal to carry out this analysis with RNA derived from the retina before its degeneration, but this tissue was not available from affected individuals. However, a substantial minority of the genes already known to be mutated in retinitis pigmentosa, including *RPGR*, *RP2*, *IMPDH1*, *PRPF31*, *PRPF8* and *PRPF3* (refs. 7, 8, 9, 10, 11, 12), are expressed throughout the body, so we reasoned that low-abundance messages from some as-yet-unidentified genes might be detected by analyzing lymphoblast mRNA.

We focused our study on 13 unrelated families with recessive retinitis pigmentosa. The families were previously screened, with negative results, for mutations in all or all but one of the following genes associated with recessive retinitis pigmentosa: *PDE6A* (accounting for 3–4% of recessive retinitis pigmentosa cases), *PDE6B* (4–5%), *RPE65* (2%), *TULP1* (1%), *USH2A* (8%) and *CNGA1* (1%)[1]. Six of the 13 families had two or more available affected siblings: one sibship had four affected members, and five sibships each had two affected members. We preferred such multiplex sibships for this study, because if any of them had recessive mutations leading to low-abundance messages, the low-abundance mRNA would be shared by all affected siblings and would thus be more likely to be recognized as distinct from fortuitously low-abundance messages from genes other than the disease gene. We created lymphoblast cell lines from peripheral blood lymphocytes from the 13 index individuals, their available affected siblings and some unaffected relatives. We also prepared lymphoblast lines from four control individuals: two

were unrelated individuals with Usher syndrome who were homozygous for the *USH2A* mutation 2299delG (E767SfsX21), one was an individual with dominant retinitis pigmentosa and the 68C>A (P23H) mutation of the rhodopsin gene, and one was an individual without retinitis pigmentosa or a family history of retinitis pigmentosa.

Lymphoblast mRNA was labeled and hybridized to an Affymetrix U133 Plus 2.0 array, which has one or more sets of oligomer probes to individually assay over 47,000 human transcripts derived from approximately 39,500 genes or ESTs. Normalized signal intensities indicate the relative amount of mRNA bound to each probe. Applying stringent filtering criteria to the numerous genes in each lymphoblast line for which the microarray analysis indicated low mRNA levels, we identified 3 to 25 candidate genes or ESTs in each family, for an aggregate of 50 candidates in the multiplex families (Supplementary Table 1). We individually eliminated 13 of the 50 corresponding genes as likely disease-associated genes because we found no mutations by direct sequencing or because our analysis of intragenic polymorphisms showed that the alleles did not segregate with disease among the relatives of the relevant index individual (Supplementary Table 2). Analysis of the remaining candidate genes has not been completed.

We concurrently evaluated the microarray results from seven families in which only one affected individual provided a blood sample for a lymphoblast line. *IDH3B* (ref. 13) was the most notable gene from those families, as the three probe sets (210014_x_at, 210418_s_at and 201509_at) that hybridized to the *IDH3B* transcript in index individual 003-053 gave normalized hybridization signals of 62, 25 and 68, respectively; in contrast, the signals in the 24 other samples were 384 ±44, 279 ±37 and 200 ±15.4 (mean ±s.d.), respectively (Fig. 1). Index individual 003-053 had an affected brother who had previously donated a blood sample for DNA analysis but had since died at age 71 years from carotid artery occlusion; lymphoblast cell lines had not been generated from this brother. Sequencing of *IDH3B* revealed that both the index individual and her deceased, affected brother were homozygous for a 1-bp deletion in codon 197 (589delA; p.I197fs; m.p.I163fs (m.p., mature protein); cDNA numbering is based on mRNA variant 1, which is the longest of the three known transcripts). The frameshift produced a premature stop codon in all three mRNA variants transcribed from *IDH3B* (Fig. 2). The parents of the affected individuals were first cousins. Four of the unaffected siblings were heterozygous, and one was wild type (Fig. 3).

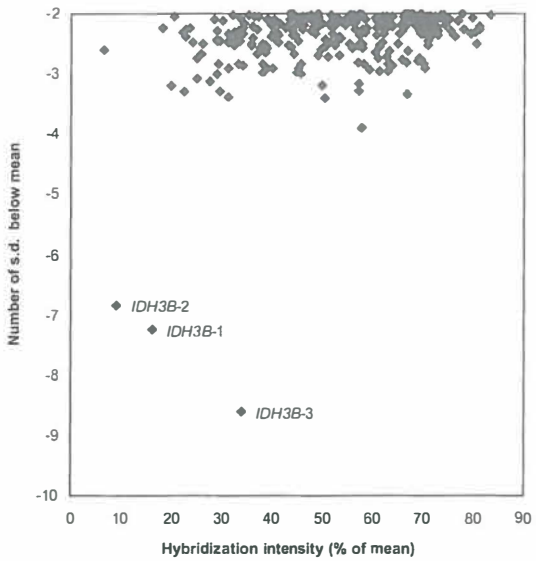


Figure 1. Scatter plot of probe-set hybridization intensities from individual 003-053. Data are organized according to percentage of respective mean signal across all subjects (x axis) and number of s.d. below this mean (y axis). The plot shows the 306 probe sets detecting hybridization signals two or more s.d. below the mean of all other subjects and controls. Of the 306 probe sets, 3 were clear outliers, with hybridization intensities more than 6 s.d. below the respective means; the 3 probe sets detected IDH3B (IDH3B-1, probe set 210014_x_at; IDH3B-2, 210418_s_at; IDH3B-3, 201509_at.) These were the only probe sets in the microarray designed to hybridize to mRNA from IDH3B.

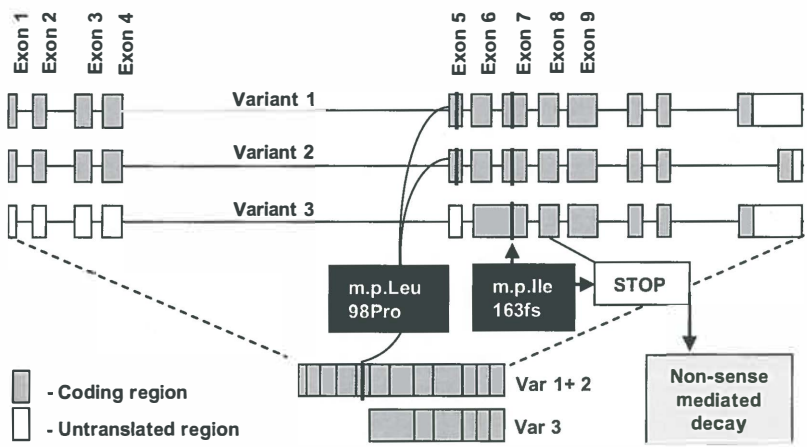


Figure 2. *IDH3B* showing three known RNA splicing variants (see URLs section below) and the location of the m.p.Ile163fs and m.p.Leu98Pro mutations. The m.p.Leu98Pro mutation is a missense mutation and alters *IDH3B* in variants 1 and 2. There is no predicted effect on variant 3 since the mutation is located in the 5' untranslated region of that variant. Splice variant 3 is missing exons 1-5 which encode 132 amino acid residues out of 385 in the full-length protein. It is not likely to encode a functional beta subunit that could compensate for the defective m.p.Leu98Pro mutation in the two other variants. The m.p.Ile163fs mutation causes a frameshift with a premature stop codon in all three variants presumably leading to mRNA degradation by nonsense-mediated decay.

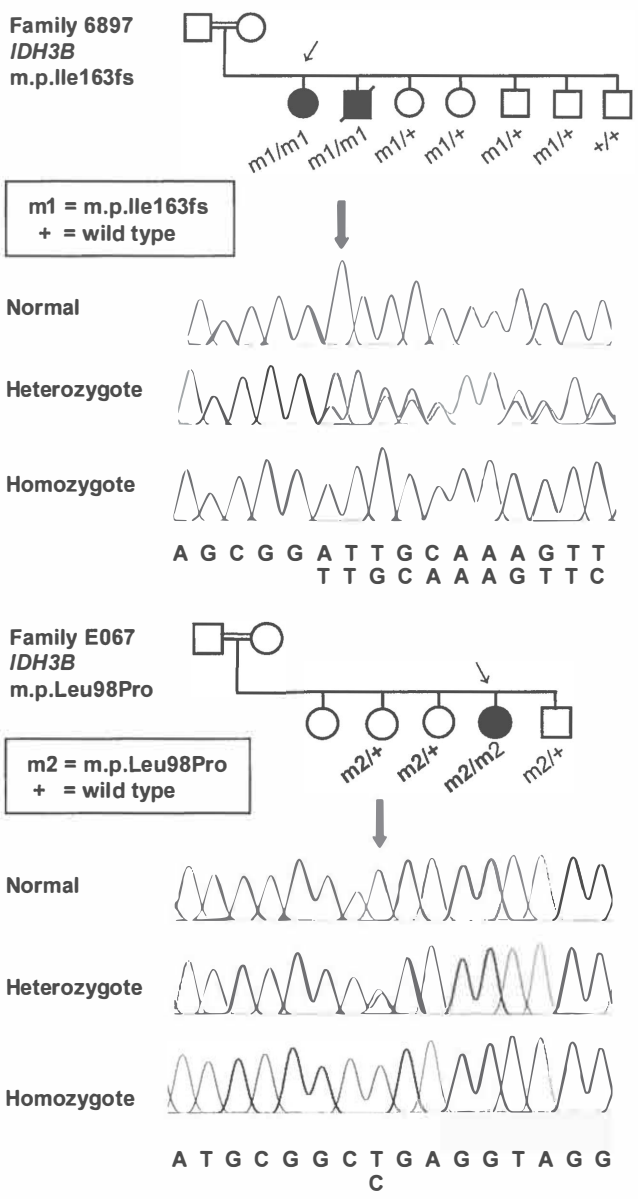


Figure 3
Pedigrees with the *IDH3B* m.p.Ile163fs and m.p.Leu98Pro mutations. Small arrows point to the index patients. Beneath each family member's symbol is that person's *IDH3B* genotype; family members for which no genotype is shown did not submit a blood sample for analysis. Fat arrows indicate the location of the mutation in the DNA sequence tracings. Nucleotides corresponding to the normal sequence (top line) and mutated sequences (lower line) are indicated below the sequence alignments.

We subsequently sequenced all 12 exons of *IDH3B* in 261 individuals with recessive retinitis pigmentosa and 285 individuals with simplex retinitis pigmentosa (most individuals with simplex retinitis pigmentosa have autosomal recessive disease). Individual 003-178 was homozygous for the m.p.L98P missense mutation (395T>C; p.L132P) in exon 5 (Fig. 2). This individual's parents were first cousins. The individual's three siblings, all unaffected, were heterozygous (Fig. 3). Neither the m.p.L98P mutation nor the m.p.I163fs mutation was found after screening leukocyte DNA from 95 control individuals without retinitis pigmentosa.

Individuals 003-053 and 003-178 were ophthalmologically examined at ages 47 and 38 years, respectively. Both had subnormal visual acuities, concentrically constricted visual fields, fundi typical of retinitis pigmentosa (pale optic discs, attenuated arterioles and intraretinal pigment deposits), impaired dark adaptation and reduced electroretinogram amplitudes indicating substantial loss of rod and cone photoreceptor function (see Supplementary Note for clinical details). They reported no other relevant health problems and, in particular, no problems typically associated with mitochondrial dysfunction (such as reduced muscle strength, cardiac dysrhythmias or reduced athletic stamina).

NAD-IDH is a heterotetramer with two α-subunits, one β-subunit and one γ-subunit[14]. In individual 003-053, who had the m.p.I163fs mutation, the β-subunit is presumably poorly expressed (as indicated by the low message levels detected by the microarray analysis). Any *IDH3B*-encoded protein produced would be truncated and unlikely to participate in a functional heterotetramer. To confirm this prediction and determine the effect of the substitution m.p.L98P, we evaluated NAD-IDH activity in lymphoblast cell lysates from the index individuals, some of their heterozygous siblings and unaffected individuals. The NAD-IDH activities in homozygous cell lysates under standard assay conditions were only 4.8% (m.p.I163fs) and 2.4% (m.p.L98P) of normal control activity, whereas activities in heterozygous cell lysates were 24% and 82%, respectively (Fig. 4). The K_m for NAD in homozygous lysates was 267- to 298-fold higher than normal, whereas the K_m in heterozygous lysates was 4- to 8-fold higher than normal (Fig. 4). The concentration of NAD in mammalian tissues has been estimated at 0.4–0.8 mM (ref. 15), which is sufficient for normal NAD-IDH (K_m for NAD ~40 μM) but too low to appreciably bind to the mutant NAD-IDH of homozygous lysates (K_m 11–12 mM).

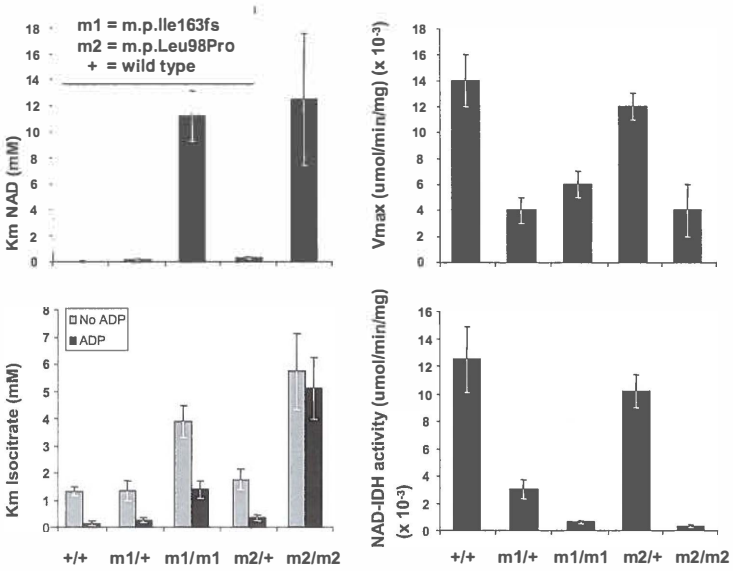


Figure 4. Activity of NAD-dependent isocitrate dehydrogenase. Average results of at least duplicate measurements with standard deviations of Km for NAD and Km for isocitrate (with or without ADP), Vmax and NAD-IDH activity under standard conditions in lymphoblast lysates from three normal subjects (+/+); two heterozygotes (m1/+) and one homozygote (m1/m1) with *IDH3B*-m.p.Ile163fs; one heterozygote (m2/+) and one homozygote (m2/m2) with the *IDH3B*-m.p.Leu98Pro mutation. The mutant homozygotes have a 267-298-fold increase in the Km for NAD (upper left panel) and a resulting loss of NAD-IDH activity (lower right panel). The normal effect of ADP in lowering the Km for isocitrate is partially (for m1 = m.p.Ile163fs) or completely (for m2 = m.p.Leu98Pro) lost in homozygote patients (lower left panel).

Without ADP, the K_m for isocitrate was three- to four-fold higher than normal in homozygous cell lysates, whereas it was normal in heterozygous cell lysates (Fig. 4). ADP is an allosteric activator for IDH3, lowering its K_m for isocitrate[16]. In our assays on lysates from unaffected controls, ADP decreased the K_m for isocitrate to 11% of that in the absence of ADP, whereas in m.p.I163fs homozygous samples ADP only decreased the K_m for isocitrate to 35% of the value in its absence. This allosteric effect of ADP was almost completely lost in homozygous m.p.L98P mutant cells: ADP only lowered the K_m for isocitrate to 88% of the value obtained in its absence (Fig. 4). The importance of the region around m.p.L98P for activation of IDH by ADP is supported by previous studies of an engineered substitution of the neighboring residue m.p.Arg99 (p.Arg133). The mutant m.p.R99Q, like the m.p.L98P mutant, lacks an allosteric effect of ADP[17].

In the archetypical model of the citric acid cycle, also known as the Krebs cycle, NAD-IDH catalyzes the conversion of isocitrate to α-ketoglutarate, an essential reaction of the cycle that simultaneously changes a molecule of NAD^+ to NADH[18]. The NADH produced in this step and other steps of the citric acid cycle is used

to generate ATP, a molecule universally used in cells as an energy source. It is therefore noteworthy that the individuals with homozygous *IDH3B* mutations had no evident health problems except for retinitis pigmentosa. Another mitochondrial enzyme, NADP-IDH, also converts isocitrate to α-ketoglutarate, but uses the cofactor $NADP^+$ instead of NAD^+. NADP-IDH activity and K_m were normal in the individuals with homozygous *IDH3B* mutations (Supplementary Table 3). The NADPH produced by NADP-IDH can be converted into NADH through the action of nicotinamide-nucleotide transhydrogenase, which is present in the inner membrane of mitochondria[19, 20]. The activity of NADP-IDH exceeds that of NAD-IDH in mammalian mitochondria[20], and the activity of the transhydrogenase is comparable to the rate of NADPH production by NADP-IDH. It is possible that, in the individuals with homozygous *IDH3B* mutations, NADP-IDH together with the transhydrogenase adequately substitute for the defective function of NAD-IDH in all tissues except the retina. In fact, our review of serial analysis of gene expression (SAGE) tags (EyeSAGE database; see Methods for URL), which crudely reflect mRNA expression levels, indicates that NADP-IDH tags are more frequent than NAD-IDH tags across all human tissues except the retina and retinal pigment epithelium (Fig. 5).

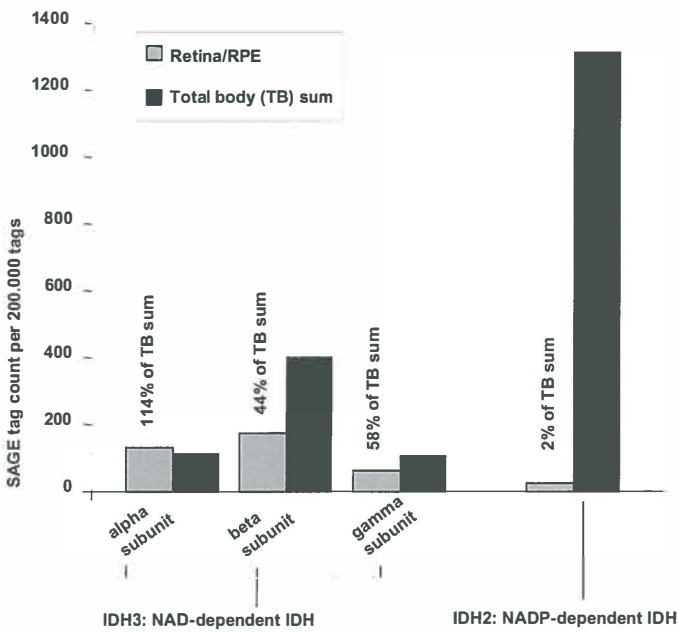


Figure 5. Frequency of SAGE tags from IDH3 and IDH2 in the retina and retinal pigment epithelium (retina/RPE) in comparison to that from all other human tissues using EyeSAGE database. IDH3: mitochondrial NAD-dependent isocitrate dehydrogenase, alpha subunit - *IDH3A* (gene ID 3491), beta subunit - *IDH3B* (gene ID 3420), gamma subunit - *IDH3G* (gene ID 3421); IDH2: mitochondrial NADP-dependent IDH - *IDH2* (gene ID 3418). The low ratio of tags from NADP-IDH suggest that the retina/RPE has a relatively low abundance of NADP-IDH and may be unusually dependent on NAD-IDH.

An alternative explanation for the viability of humans with greatly reduced NAD-IDH is that NADP-IDH may actually be the principal catalyst of the isocitrate to α-ketoglutarate reaction in the citric acid cycle in all tissues, whereas NAD-IDH serves as an accessory enzyme that augments or regulates the reaction, with the retina being particularly sensitive to the loss of this accessory activity. In this regard, it is notable that the K_m for isocitrate is about 1 μM for NADP-IDH, which is low relative to the 40–60 μM of isocitrate estimated to be present in mammalian mitochondria[21]. Thus, NADP-IDH would normally be saturated with isocitrate. Furthermore, the K_m for NADP is ~2 μM, whereas mammalian tissue levels of NADP are estimated at ~100 μM (ref. 15), so the coenzyme site would also be saturated. In contrast, the K_m of NAD-IDH for isocitrate is 1–2 mM (in the absence of ADP), which is high relative to the mitochondrial levels of isocitrate. It is only in the presence of ADP that the K_m for isocitrate is lowered to the 0.1-mM range, thereby allowing that enzyme to make a contribution to the oxidative decarboxylation of isocitrate. These considerations all support the interpretation that NADP-IDH is the major enzyme serving this step of the citric acid cycle, with the retina being a possible exception. The association of loss-of-function mutations with retinitis pigmentosa suggests that NAD-IDH is essential for normal retinal function. Further studies of mitochondrial metabolism in the retina and other tissues should help to confirm the association of NAD-IDH mutations with retinitis pigmentosa and the role of the IDH family of enzymes in mitochondrial metabolism.

Methods

Subjects

Twenty-one individuals with recessive retinitis pigmentosa from 13 unrelated families and four control individuals were included in the study. This study adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Boards of the Massachusetts Eye and Ear Infirmary and Harvard Medical School and the Human Subjects Review Board of the University of Delaware. Informed consent was obtained from all study participants. See Supplementary Note for further details regarding subject recruitment and phenotyping criteria.

Lymphoblast cell lines

Cell lines were created from whole blood by immortalization with Epstein-Barr virus. Cells were grown to confluence, pelleted, suspended in TRIzol (Invitrogen) and stored at -80 °C until RNA was extracted.

RNA isolation, labeling and hybridization

Details in addition to those indicated in the text are provided in Supplementary Methods.

Statistical analysis

Raw data reflecting hybridization intensities from individual probes of 25 different chips were provided as Microarray Suite 5.0 (Affymetrix) experiment data files (.chp, .txt, .rpt, .exp and .cel) and were normalized to a chip with median overall brightness using dChip[22]. The normalized data were extracted into a Microsoft Excel file, and hybridization intensities from all probes of the affected members of individual families were compared to all of the other samples. Significance in multiplex families was tested using a one-tailed Student's *t*-test assuming unequal variance, a Bonferroni correction and $P < 0.05$. In families with only one affected individual available, the transcript in that individual had to have a hybridization signal more than two s.d. below the mean hybridization intensity found in all other subjects.

DNA sequencing of candidate genes

Sequencing was done by amplifying individual exons along with flanking intron sequences using primers designed with Primer 3 (see below for URL). The amplified fragments were treated with ExoSAP-IT (US Biochemical) to eliminate unincorporated primers and dNTPs and then directly sequenced using BigDye version 3.1 and an ABI 3100 automated sequencer (Applied Biosystems). For some genes, only exons with one or more known SNPs were amplified and sequenced to determine whether the alleles segregated with disease in the relevant families. Primer sequences are listed in Supplementary Table 4.

Protein extraction

Extraction was done by resuspending each lymphoblast cell pellet from a 200-ml culture (see Supplementary Methods for details of lymphoblast culture) in 1 ml of mammalian protein extraction reagent (Pierce). The volume of each cell pellet ranged from 300 to 400 μl and yielded between 9 and 15 mg of protein. The cells were incubated on ice during lysis for 1 h. The lysate was then centrifuged, and the supernatant was used for measuring enzyme activity and protein concentration. Protein concentration was determined from an absorbance at 280 nm after correcting for the ratio of the absorbances at 280 and 260 nm (ref. 23).

Activity of NAD-IDH and NADP-IDH

Activity was determined using the Perkin-Elmer fluorescence spectrophotometer MPF-3 by monitoring the time-dependent increase in the fluorescence of NADH

or NADPH at 440 nm after excitation at 340 nm. Standard assay conditions for NAD-IDH were 33 mM Tris-acetate buffer (pH 7.2), 20 mM DL-isocitrate, 1 mM NAD and 1 mM $MnSO_4$ (final concentrations). Kinetic studies were conducted by varying the concentration of one substrate at a time. Measurements of the K_m for isocitrate in the absence and presence of 1 mM ADP were done at 10 mM NAD. Standard assay conditions for NADP-IDH were 30 mM triethanolamine hydrochloride buffer (pH 7.4), 4 mM DL-isocitrate, 0.1 mM NADP and 2 mM $MnSO_4$. The K_m values for isocitrate and for NADP were determined by varying the concentration of each substrate at otherwise standard conditions.

URLs

RetNet, http://www.sph.uth.tmc.edu/retnet/; EyeSAGE, http://neibank.nei.nih.gov.ezp-prod1.hul.harvard.edu/EyeSAGE/index.shtml; IDH3B splicing variants, http://genome.ucsc.edu/cgi-bin/hgTracks?position=chr20:2587041-2592843&hgsid=107503458&refGene=pack&hgFind.matches=NM_006899; Primer3, http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi.

Accession codes

Gene Expression Omnibus: mRNA expression data have been deposited with accession code GSE12086. Entrez Nucleotide for IDH3B (chromosome 20p13): NM_006899 (transcript variant 1), NM_174855 (transcript variant 2) and NM_174856 (transcript variant 3). Entrez Gene: 3491 (IDH3A, α-subunit), 3420 (IDH3B, β-subunit), 3421 (IDH3G, γ-subunit) and 3418 (IDH2). OMIM for IDH3B: 604526.

References

1. Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. Lancet. 2006;368:1795–1809.
2. Daiger SP. Identifying retinal disease genes: how far have we come, how far do we have to go? Novartis. Found. Symp. 2004;255:17–27.
3. Losson R, Lacroute F. Interference of nonsense mutations with eukaryotic messenger RNA stability. Proc. Natl. Acad. Sci. U. S. A. 1979;76:5134–5137.
4. Noensie EN, Dietz HC. A strategy for disease gene identification through nonsense-mediated mRNA decay inhibition. Nat. Biotechnol. 2001;19:434–439.
5. Huusko P, et al. Nonsense-mediated decay microarray analysis identifies mutations of EPHB2 in human prostate cancer. Nat. Genet. 2004;36:979–983.
6. Dybbs M, Ngai J, Kaplan JM. Using microarrays to facilitate positional cloning: identification of tomosyn as an inhibitor of neurosecretion. PLoS. Genet. 2005;1:6–16.
7. Meindl A, et al. A gene (RPGR) with homology to the RCC1 guanine nucleotide exchange factor is mutated in X-linked retinitis pigmentosa (RP3). Nat. Genet. 1996;13:35–42.
8. Schwahn U, et al. Positional cloning of the gene for X-linked retinitis pigmentosa 2. Nat. Genet. 1998;19:327–332.
9. Gu JJ, Spychala J, Mitchell BS. Regulation of the human inosine monophosphate dehydrogenase type I gene. Utilization of alternative promoters. J. Biol. Chem. 1997;272:4458–4466.
10. Vithana EN, et al. A human homolog of yeast pre-mRNA splicing gene, PRP31, underlies autosomal dominant retinitis pigmentosa on chromosome 19q13.4 (RP11). Mol. Cell. 2001;8:375–381.
11. McKie AB, et al. Mutations in the pre-mRNA splicing factor gene PRPC8 in autosomal dominant retinitis pigmentosa (RP13). Hum. Mol. Genet. 2001;10:1555–1562.
12. Chakarova CF, et al. Mutations in HPRP3, a third member of pre-mRNA splicing factor genes, implicated in autosomal dominant retinitis pigmentosa. Hum. Mol. Genet. 2002;11:87–92.
13. Kim YO, et al. Assignment of mitochondrial NAD(+)-specific isocitrate dehydrogenase beta subunit gene (IDH3B) to human chromosome band 20p13 by in situ hybridization and radiation hybrid mapping. Cytogenet. Cell Genet. 1999;86:240–241.
14. Ramachandran N, Colman RF. Chemical characterization of distinct subunits of pig heart DPN-specific isocitrate dehydrogenase. J. Biol. Chem. 1980;255:8859–8864.
15. Dolphin D, Poulson R, Avramovic O. Pyridine Nucleotide Coenzymes (Part B), Coenzymes and Cofactors. Wiley-Interscience Publication; New York: 1987.
16. Cohen PF, Colman RF. Diphosphopyridine nucleotide dependent isocitrate dehydrogenase from pig heart. Characterization of the active substrate and modes of regulation. Biochemistry. 1972;11:1501–1508.
17. Soundar S, Park JH, Huh TL, Colman RF. Evaluation by mutagenesis of the importance of 3 arginines in alpha, beta, and gamma subunits of human NAD-dependent isocitrate dehydrogenase. J. Biol. Chem. 2003;278:52146–52153.
18. Garrett RH, Grisham CM. Biochemistry. 2004 Brooks Cole.
19. Hoek JB, Rydstrom J. Physiological roles of nicotinamide nucleotide transhydrogenase. Biochem. J. 1988;254:1–10.
20. Moyle J, Mitchell P. The proton-translocating nicotinamide-adenine dinucleotide (phosphate) transhydrogenase of rat liver mitochondria. Biochem. J. 1973;132:571–585.
21. Smith CM, Plaut GW. Activities of NAD-specific and NADP-specific isocitrate dehydrogenases in rat-liver mitochondria. Studies with D-threo-alpha-methylisocitrate. Eur. J. Biochem. 1979;97:283–295.
22. Li C, Hung WW. Model-based analysis of oligonucleotide arrays: model validation, design issues and standard error application. Genome Biol. 2001;2 research0032.
23. Warburg O, Christian W. Biochem. Z. 1941:384–387.

Supplementary material

Supplementary Table 1.
Number of candidate genes resulting from microarray analysis.

	Family					
	1	2	3	4	5	6
Genes or ESTs with only one probe on the microarray and for which (1) the microarray indicated an mRNA level < 50% of the average of all other samples, (2) the low mRNA level among the affected siblings was statistically significantly different from the other samples, and (3) the raw expression level was at least 100 arbitrary units (normal dynamic range is 5-14,000) in at least one sample (to eliminate transcripts with an expression level barely above noise for the microarray)	1	2	7	3	11	1
Genes or ESTs with 2 or more probes in the microarray and for which (1) only one of the probes indicated an mRNA level < 50%, (2) the low mRNA level among the affected siblings was statistically significantly different from the other samples, (3) the raw expression level was > 100 for the probe showing a significant reduction in mRNA level.	2	0	0	0	6	0
Genes or ESTs which had 2 or more probes on the microarray and for which 2 or more probes indicated a significantly reduced mRNA level in the affected siblings in comparison with the other samples (independent of signal level or amount of reduction)	2	2	3	1	8	2
Total	**4**	**4**	**10**	**4**	**25**	**3**
Number of candidate genes/clones that were screened by direct sequencing or segregation analysis.	2	1	3	0	5	2

Applying stringent filtering criteria to the numerous genes in each lymphoblast line for which the microarray analysis indicated low mRNA levels, we narrowed our analysis to between 3 to 25 candidate genes or ESTs for each multiplex family.

Supplementary Table 2.
Analysis of candidate genes in multiplex families.

Gene	Chrom	Exons	Screened		
Family 1					
ZEBD3	5	3	Exon 3		No segregation with disease
Clone AF271776	M	1	1	No mutations found	Segregation uninformative
Family 2					
DKFZP434C171	5	9	9	No mutations found	
Family 3					
TRA2A	7	8	8	No mutations found	Segregation uninformative
TNRFSF7	12	6	6	No mutations found	Segregation uninformative
Clone IMAGE:111693	16	1	1	No mutations found	Segregation uninformative
Family 5					
RGS16	1	5	5	No mutations found	
BICD1	12	10	1		No segregation with disease
PEX6	6	17	1		No segregation with disease
Clone IMAGE:4794726	7	1	1	No mutations found	
Clone IMAGE:3443353	1	1	1	No mutations found	
Family 6					
GBP1	1	11	9		No segregation with disease
clone BRACE-2020028	1	1	1		No segregation with disease

Candidate genes from micro-array analysis in multiplex families which were analyzed either by direct DNA sequencing or by analysis of linked polymorphisms to determine if alleles cosegregated with disease in the respective families. Results from these genes made it unlikely that any of them was responsible for RP.

Supplementary Table 3.
Enzyme activity for NADP-dependent isocitrate dehydrogenase.

Type	Km NADP (uM)	Km Isocitrate (uM)	Vmax (umol/min/mg)	Specific activities[a] (umol/ min/ mg)
Normal	1.92 ± 0.22	1.36 ± 0.74	47 ± 5 (x 10-3)	47 ± 5 (x 10-3)
Heterozygote c.589delA	2.52 ± 0.38	1.55 ± 0.11	56 ± 3 (x 10-3)	53 ± 3 (x 10-3)
Homozygote c.589delA	3.39 ± 0.76	0.59 ± 0.19	57 ± 2 (x 10-3)	60 ± 2 (x 10-3)
Heterozygote m.p.Leu98Pro	2.01 ± 0.76	0.55 ± 0.13	25 ± 1 (x 10-3)	33 ± 1 (x 10-3)
Homozygote m.p.Leu98Pro	2.01 ± 0.76	1.44 ± 0.37	44 ± 1 (x 10-3)	41 ± 1 (x 10-3)

[a] Specific activities were determined in tris acetate buffer, pH 7.4, 20mM Isocitrate, 1mM NADP and 1mM $MnSO_4$.

Average results of Km for NADP and Km for isocitrate and Vmax in cell lines of three normal subjects; two heterozygote and one homozygote *IDH3B*- m.p.Ile163fs; one heterozygote and one homozygote m.p.Leu98Pro subject. The Kms for NADP as well as isocitrate are similar in all the samples. (Note that the NADP-activities reflect the sum of mitochondrial NADP-IDH (IDH2) and cytosolic NADP-IDH (IDH1) activities. Human EST profiles (available from www.**ncbi**.nlm.nih.gov) suggest that IDH1 and IDH2 are approximately equally well expressed in lymphocytes and lymph nodes. In the eye, the expression level of the mitochondrial IDH2 appears to be about 2.5 times higher than is the cytosolic IDH1. It is reasonable to assume that the mitochondrial NADP-IDH (IDH2) would be the enzyme participating in the citric acid cycle since all the other citric acid cycle enzymes, as well as the electron transport system, are localized within the mitochondria.)

Supplementary Table 4.
Primer sequences and conditions used for amplification of IDH3B.

Exon	Direction	Primer sequence	Annealing Temperature
1 + 2	Sense	CTCAGTACAGGCCGGAAGTC	60
	Antisense	CACGGATCCTGGGAGTAGAG	
3 + 4	Sense	CCGGGTCTCCCTACTTCTCT	58
	Antisense	TGTGGGGAGAAGGGCTTAAT	
5 + 6	Sense	CTGATGTGGGAATGGGAGAT	58
	Antisense	GCAGCAGCTAGGTGACACTG	
6 + 7	Sense	TGGGTATATGACTCGGCACA	56
	Antisense	GGGACAGAATCAGCCAAGAA	
8 + 9	Sense	GGCATGGCTTTGTGTAGGAT	56
	Antisense	GGAGATATTGGGATGGGAGA	
10 + 11	Sense	CCACCATGCCTCTTTTCAGT	58
	Antisense	AGGTTCCCCAGAGGGTAGTG	
12	Sense	TGGCTTCCATTTCCTCAATC	56
	Antisense	TACCATAGCCCAAGGTGACA	

Primer sequences and conditions that were used for the amplification of the 12 *IDH3B* exons with flanking intron sequences.

Supplementary Methods

Lymphoblast cell lines. Lymphoblasts were immortalized and grown at the Harvard Partners Center for Genetics and Genomics. Prior to harvesting for isolating mRNA, cells were grown to confluence in T25 flasks (10 ml medium/ flask), and then put in T75 flasks with an additional 15 ml of fresh medium. When the cells reached confluence (typically after 3 - 6 weeks), another 25 ml of medium was added, for a total of 50 ml. The cells were harvested 24 hours afterwards. Cells were pelleted by centrifugation at room temperature and washed twice with PBS buffer. Finally, cells were pelleted again, suspended in Trizol, divided into 4 aliquots, and kept at -80 C. Cell counts indicated that each aliquot contained about 2.5-17.5 million cells, although cell counts were not performed on some of the harvested lymphoblast cultures.

Larger quantities of cells were required to measure IDH activity from lymphoblasts. From each cell line, 10 flasks of cells were grown to confluence, with 200 mls of liquid media per flask. Cells were pelleted by centrifugation, washed with PBS, re-pelleted and flash-frozen in liquid nitrogen and stored at -80 C. Each of the 10 flasks yielded a cell pellet with a volume of approximately 300-400 ul.

RNA isolation, labeling and hybridization An aliquot of the lymphoblast-trizol suspension was thawed and RNA was purified using the Promega SV Total RNA Isolation kit and resuspended in RNAse-free water to a final concentration ranging from 0.2 to 0.7 μg/μl. The RNA yield from the different cell lines was approximately 5-18 μg. cDNA was synthesized and biotin-labeled from 1.5-2.0 μg total RNA/ sample by priming with random decamers and using MMLV reverse transcriptase according to standard protocols. The cDNA was used as template to produce biotin-labeled cRNA which was hybridized to the Affymetrix gene chip "Human Genome U133Plus2.0" containing probes from all known human genes and EST transcripts. The microarrays were washed and the hybridizing biotin-labeled cRNA was detected with a streptavidin-conjugated fluorophore. The hybridized chips were scanned and analyzed with Microarray Suite (MAS) 5.0 (Affymetrix) (performed at the Dana-Farber Cancer Institute Microarray Center, Boston, MA). The probes (each 25 bases in length) detect over 47,000 transcripts from approximately 39,500 human genes and ESTs.

To enrich for transcripts with low expression due to recessive mutations (and not due to normal variation in gene expression)[1], we confined our search for mutations to transcripts that met the following criteria. 1) the hybridization intensity was at least 100 units (out of a range of 5-14,000) in at least one of the

subjects to eliminate transcripts normally expressed in lymphocytes at a low level indistinguishable from background noise; 2) the probe sets detecting the transcript showed a mean hybridization intensity in the affected family members less than 50% of the mean intensity found in all other samples; 3) genes where more than one corresponding probe showed significant results were evaluated irrespective of hybridization intensity or amount of reduction; 4) the transcript had comparably low hybridization intensity in all evaluated affected siblings of the index patient; 5) in multiplex families, the hybridization signal for the transcript was statistically significantly lower than the signal from all other patients and controls even after a Bonferroni correction for the thousands of transcripts evaluated; 6) in families with only one available patient, the transcript in that patient had a hybridization signal more than two standard deviations below the mean hybridization intensity in all other patients. As many as 205 to 473 probes (34 for the sibship with 4 affected members) were significantly decreased in the tested family in comparison to the other samples. Of these, as many as 43 to 121 probes (8 for the sibship with 4 affected members) had a > 50% reduction in average expression compared to the average expression of all other samples (Supplementary Table 1). After applying the filtering criteria, there were a total of 3 to 25 candidate genes per family (50 in total) as is indicated in the original article (Supplementary Table 1). Two of the four controls were homozygotes with the previously identified USH2A mutation (Glu767SerfsX21 (c.2299delG)) which results in a premature termination codon. The mutant mRNA would be expected to suffer nonsense-mediated decay. Unfortunately, USH2A transcripts hybridized very weakly with the microarray probes for this gene, with hybridization intensities indistinguishable from background noise in all subjects. Thus, the microarray data did not reveal whether the nonsense mutation affected mRNA levels.

DNA sequencing of the *IDH3B* gene. Six primer pairs were selected to cover the 12 exons and flanking intron regions. For primer sequences and amplification conditions used see Supplementary Table 4.

1. Yan,H., Yuan,W., Velculescu,V.E., Vogelstein,B., & Kinzler,K.W. Allelic variation in human gene expression. *Science* 297, 1143 (2002).

Supplementary Note

Patient recruitment and phenotyping criteria:
The patients described in this study were selected from a group of patients who had come to the ERG service at the Massachusetts Eye and Ear Infirmary to be evaluated for retinal degenerations and who had donated leucocyte DNA as part of a general study to identify genetic mutations causing retinitis pigmentosa (RP). All patients had been diagnosed with retinitis pigmentosa based on elevated final dark adaptaton thresholds, reduced and delayed ERGs (electroretinograms), retinal arteriolar narrowing and bone spicule pigmentation in most cases. All selected for the present study had autosomal recessive disease based on family history. Of the 250 identified unrelated patients with recessive RP, we selected 13 who were previously screened with negative results for mutations in all or all but one of the following known recessive RP-causing genes: PDE6A, PDE6B, RPE65, TULP1, USH2A, and CNGA1. After obtaining informed consent, these 13 patients, 8 of their affected siblings and 4 control individuals (three with RP due to known gene defects, and one unaffected person) were asked to provide a fresh blood sample for the generation of the lymphoblast line.

The specific clinical ophthalmological findings of the patients with the *IDH3B* mutation described in our study were as follows: Best-corrected visual acuities were 20/40 in the right eye (OD) and 20/30 in the left eye (OS) in patient 003-053 and 20/25 in both eyes (OU) in patient 003-178. The visual field was constricted concentrically to a diameter in each eye of 20° (003-053) and 20-30° (003-178) (normal ≈ 135° horizontally and 90° vertically) in each eye with a V-4e white test light in the Goldmann perimeter. Both patients retained a crescent of vision in the inferior far periphery. Patient 003-053 had early posterior subcapsular cataracts whereas patient 003-178 had clear lenses. Both index patients had waxy pallor of the optic discs, attenuated retinal arterioles, and moderate to heavy intraretinal pigment in a bone-spicule configuration in the periphery. After 30 minutes of darkness, the ability of the patients to detect dim light was impaired. Specifically, they required light 1000 times brighter than normal for minimal perception (i.e., final dark adaptation thresholds were elevated 3 log units). Mixed cone-rod ERG responses to 0.5 Hz white flashes were 9.8 μV OD and 22.1 μV OS in 003-053 and 17.6 OU in 003-178 (normal ≥ 350 μV). Cone isolated responses to 30 Hz white flicker were 4.7 μV OD and 9.4 μV OS in 003-053 and 2.3 μV OU in 003-178 (normal ≥ 50 μV). These subnormal ERG amplitudes reflect substantial loss of rod and cone photoreceptor function and are characteristic of RP.

Chapter 3

Search for a correlation between telomere length and severity of retinitis pigmentosa due to the dominant rhodopsin Pro23His mutation

Dyonne T. Hartong,[1,3] Terri L. McGee,[1,2] Michael A. Sandberg,[2]
Eliot L. Berson,[2] Folkert W. Asselbergs,[4] Pim van der Harst,[4]
Immaculata De Vivo,[5] and Thaddeus P. Dryja[1]

[1]Ocular Molecular Genetics Institute, Harvard Medical School, Massachusetts Eye and Ear Infirmary, Boston, MA, USA
[2]The Berman-Gund Laboratory for the Study of Retinal Degenerations, Harvard Medical School, Massachusetts Eye and Ear Infirmary, Boston, MA, USA
[3]Dept .of Ophthalmology, University Medical Center Groningen, Groningen, The Netherlands
[4]Dept. of Cardiology, University Medical Center Groningen, Groningen, The Netherlands
[5]Channing Laboratory, Dept. of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Mol Vis. 2009; 15: 592–597.

Abstract

Purpose

Great variation exists in the age of onset of symptoms and the severity of disease at a given age in patients with retinitis pigmentosa (RP). The final pathway for this disease may involve apoptotic photoreceptor cell death. Telomere length is associated with biologic aging, senescence, and apoptosis. We evaluated whether the length of telomeres in leukocytes correlated with the severity of RP in patients with the Pro23His rhodopsin mutation who have shown marked heterogeneity in disease severity.

Methods

We evaluated 122 patients with the Pro23His rhodopsin mutation. The patients' retinal function was stratified according to their 30-Hz cone electroretinogram (ERG). The length of telomeres in leukocytes was measured by the quantitative real time polymerase chain reaction (qRT–PCR) method in the 15 patients with the highest age-adjusted 30-Hz ERG amplitudes and in the 15 patients with the lowest amplitudes.

Results

Mean leukocyte telomere length was similar in the 15 patients with the highest cone ERG amplitudes (median: 0.40 units; interquartile range 0.36–0.56) and the 15 patients with the lowest cone amplitudes (median: 0.41 units; inter quartile range 0.34 –0.64; p=0.95).

Conclusions

We found no evidence for an association between telomere length and the severity of RP as monitored by the cone ERG in patients with the Pro23His rhodopsin mutation.

Introduction

Retinitis pigmentosa (RP) is a group of inherited retinal degenerations with progressive photoreceptor cell death typically causing night blindness, constricted visual fields, and in later stages, a decrease in visual acuity. As the condition progresses, the cone electroretinogram (ERG) amplitude decreases. Mutations in the rhodopsin gene (*RHO*; OMIM ID: +180380) account for about 25% of the dominantly inherited RP cases and less than a few percent of recessively inherited cases.[1-4] The Pro23His mutation is the most frequently reported rhodopsin mutation in the United States,[5] accounting for about 8.5% of all dominant RP cases or about 1/3 of those with a dominant rhodopsin mutation.[6] Interfamilial and intrafamilial variation in disease severity among patients with this mutation have been described,[7,8] suggesting that factors besides the primary gene defect contribute to the disease.

Telomeres are structures at the ends of chromosome arms consisting of tandem repeats of the nucleotide sequence TTAGGG. These repetitive elements stabilize chromosomes by preventing fusion with other chromosome ends and by impeding degradation of coding DNA.[9-11] Short telomere length has been associated with apoptosis.[12-14] Telomere length is dependent on the number of previous cell divisions and, thus, decreases with age. This decrease is compensated in part by telomerase, which adds TTAGGG tandem repeats to the 3th end of the DNA strand.[15-19] Telomere length is highly variable among individuals, and this variation is detectable at birth.[20-22] Telomere lengths are similar in different tissues of the same individual, so the analysis of one cell type (e.g., leukocytes) reflects the telomere size throughout that individual.[23,24]

Previous studies have shown an inverse relationship between leukocyte telomere length and the occurrence of age-related diseases such as chronic heart failure[25] and dementia[26]. Shorter telomere length has also been associated with disease severity.[27]

In this study we evaluated the possible association between telomere length and the severity of RP. We hypothesized that individuals with shorter telomere lengths may have more severe photoreceptor degeneration. We evaluated 122 patients who had autosomal dominant RP due to the Pro23His mutation and selected 15 patients with the best preserved retinal function and the 15 who had the least preserved retinal function. Telomere length was compared with the loss of retinal function as indicated by the amplitude of the 30-Hz cone ERG.

Methods

Patient selection

This study conformed to the Declaration of Helsinki and was approved by the Institutional Review Boards of Harvard Medical School and the Massachusetts Eye and Ear Infirmary and the Human Subjects Committee of the Harvard School of Public Health. Informed consent was obtained from all patients. We evaluated 122 RP patients previously found to have the dominant mutation Pro23His in *RHO*.[28] Patients were recruited from the files of the Berman-Gund Laboratory, Harvard Medical School. Patients had elevated final dark adaptation thresholds and reduced rod ERGs; all had attenuated retinal arterioles and most had intraretinal bone spicule pigment around the periphery. The general health of the patients was good. Blood samples from the patients were obtained through phlebotomy and leucocyte DNA was isolated using a Phenol/Chloroform extraction.

Measurements of retinal function

Retinal function was evaluated based on the ERG amplitude recorded in response to 30-Hz white flashes. Full-field ERG responses were obtained following pupil dilation using one drop of a solution containing 10% phenylephrine hydrochloride and 1% cyclopentolate hydrochloride (Akorn, inc., Buffalo Grove, IL) and 45 min of dark adaptation. ERGs were recorded with a contact lens electrode placed on the cornea topically anesthetized with one drop of 0.5% proparacaine hydrochloride (Alcon Laboratories, Inc. Fort Worth, TX). Responses below 10 μV were recorded with narrow band-pass filtering and then computer averaged to increase the signal to noise ratio as described previously.[29,30] Amplitudes were averaged between the two eyes (when results from both eyes were available) and adjusted for age and refractive error.

Telomere lengths

The relative telomere lengths were determined using a modified quantitative real time polymerase chain reaction (qRT–PCR).[31,32] Briefly, the 7900 HT thermocycler (Applied Biosystems, Foster city, CA) was used to obtain the relative length of telomeres, expressed as the ratio between the repeat copy number of telomeres (T) and a reference single-copy gene (S; 36B4 gene, chromosome 12). All samples were compared with the same reference DNA sample. This method has been shown to correlate with Southern blot measurements of telomere length.[33,34]

For each reaction 5 ng of DNA were dried in the well of a reaction plate and resuspended in 10 μl of PCR reaction mix, which contained 1X Qiagen Quantitect Sybr Green Master Mix, 2.5 mM of dithiothreitol, and primers. Primers for the telomere reaction were 270 nM of the sense primer (GGT TTT TGA GGG TGA

GGG TGA GGG TGA GGG TGA GGG T) and 900 nM of the antisense primer (TCC CGA CTA TCC CTA TCC CTA TCC CTA TCC CTA TCC CTA). Primers for the 36B4 reaction were 300 nM of the sense primer (CAG CAA GTG GGA AGG TGT AAT CC) and 500 nM of the antisense primer (CCC ATT CTA TCA TCA ACG GGT ACA A). The temperature for the first 5 min was 95 °C; this was followed by 40 cycles consisting of 15 s at 95 °C and 2 min at 54 °C for the telomere reaction or 1 min at 58 °C for the 36B4 reaction. ABI 7900HT software calculated the cycle threshold for the Telomere (T) and reference gene (S) for each sample. The ratio T:S represented the relative amount of telomere DNA compared to single copy DNA and therefore corresponded to relative telomere length. All samples were analyzed in triplicate and the coefficient of variation (CV), describing the amount of repeat variability, was calculated.

Statistical analysis

We confined our analysis of telomere length to sets of patients at the extremes of disease severity as determined by cone 30-Hz ERG amplitudes. Individuals at the extremes for a continuously variable trait (such as disease severity) provided the most power for uncovering the responsible factors, since they are most likely to differ in the level of the responsible factors or their frequency (see, for example, power calculations for mapping genes for quantitative traits)[35]. First, the relative telomere lengths (T:S ratios) in the high and low ERG amplitude groups were compared by means of a two-tailed Mann–Whitney U test. Second, a linear regression analysis compared the $\log_e$ 30-Hz ERG amplitude to the T:S ratio adjusting for age and refractive error. The Spearman correlation was used to test the association between age and telomere length. A p-value of <0.05 was considered statistically significant. The aforedescribed calculations were performed with SPSS version 14 software (SPSS, Chicago, IL) or with JMP, version 6 (SAS Institute, Cary, NC).

Results

Patients

We ranked 122 patients with the *RHO*-Pro23His mutation according to their mean 30-Hz ERG amplitude, adjusted for age and refractive error. After excluding a few outlier patients with respect to age, we selected the 15 with the highest 30-Hz ERG amplitude and the 15 with lowest. The 15 patients with the least severe RP (i.e., those with the highest ERG amplitudes) consisted of nine males and six females. The 15 patients with the most severe disease (i.e., those with the lowest ERG amplitudes) comprised eight males and seven females. The mean age at time of phlebotomy for DNA samples from these two groups was 46.7 years (range: 27–58) and 45.1 years (range: 31–62), respectively. Many of the 122 patients were related

to others in this set. Of the 30 individuals included in the analysis of the least and most severely affected, 14 were first degree relatives (siblings). An additional nine individuals were also related to others in the analysis set but were more distantly related. Among the first degree relatives, four sets of two siblings appeared in the group with high ERG amplitudes (least severe), one set of three siblings appeared in the group with low ERG amplitudes (most severe), and one sibship was split among the groups with two siblings in the most severe group and one in the least severe group. Seven patients were not related to any of the 30 extreme patients in the in the analysis set. No unaffected controls were included since we were only interested in the variation of telomere length related to disease severity within this group of Pro23His mutants. For patient characteristics and individual results, see Table 1.

Table 1.
Patients included in high and low ERG amplitude groups.

Sample ID	Family ID	1°relative	Sex	Age (years)	Av30Hz ERG(μV)	T/S ratio
High ERG amplitude group						
218-288	5938	218-289	f	34	81.0	0.430198
001-191	1566	-	m	38	76.0*	0.537613
218-289	5938	218-288	m	41	69.5	0.557743
226-1070	6149	226-1063	m	49	67.5	0.364527
226-1063	6149	226- 1070	m	51	51.5	0.350156
226-007	5850	218-005, 218-002	f	56	50.0	0.756707
218-309	6281	-	f	46	46.0	0.376173
226-685	5970	001-299	m	56	42.5	0.397979
001-299	5970	226-285	m	58	41.1	0.341629
226-638	6149	-	f	27	41.0	0.380276
218-243	6038	226-905	m	52	38.0	0.394993
226-905	6038	218-243	f	50	38.0	0.556106
001-385	6803	-	m	46	35.3	0.519532
218-282	5938	-	m	47	27.5	0.56872
218-280	E716	-	f	49	12.6	0.311241
Low ERG amplitude group						
001-007	6994	-	f	34	2.3	0.364527
226-1426	5998	-	f	42	1.04	0.769996
001-162	6653	-	f	46	0.7	0.368992
218-003	5850	-	m	38	0.65	0.412048
001-390	1509	-	m	48	0.47	0.376173
218-005	5850	226-007, 218-002	m	55	0.3	0.608039
001-131	6281	-	m	43	0.3	0.355099
218-255	5938	001-387, 218-031	f	32	0.23	0.586807
001-089	6149	-	f	31	0.2*	0.799895
218-407	6888	-	f	62	0.17	0.274106
218-060	1566	-	f	62	0.16	0.340283
001-078	6038	-	m	49	0.15	0.197481
001-387	5938	218-255, 218-031	m	34	0.13*	0.651994
218-002	5850	226-007, 218-005	m	60	0.12	0.430762
218-031	5938	218-255, 001-387	m	41	0.08	0.638473

This table lists the 30 Hz cone ERG amplitudes averaged across both eyes (Av30Hz ERG) for the 15 patients included in the high ERG amplitude group (least severely affected) and the 15 patients included in the low ERG amplitude group (most severely affected) along with the relative telomere lengths (ie. T/S ratio) obtained for each patient, the patient's age at phlebotomy, their gender and any first degree relatives also included in this study. Age ranges, gender distribution and T/S ratios were similar between the two groups whereas the 30 Hz ERG amplitudes were on average nearly 100 fold greater in the high ERG group than the low ERG group. Amplitudes marked with an * indicate that data was available for only one eye. All patients had reduced or nondetectable rod ERG amplitudes (data not shown); some still had normal cone ERG amplitudes (normal range=50–125 μV).

Analysis of the relative telomere lengths

The coefficient of variation in our study was satisfactorily low with 1.27% for the T assay and 0.64% for the S assay. Spearman's correlation test showed a modest inverse relationship between patient age and relative telomere length in the 30 patients (r=-0.38; p=0.037). We found no significant difference in T:S ratio between the 15 patients with the highest cone ERG amplitudes (median: 0.40 units; interquartile range 0.36–0.56) and the 15 patients with the lowest cone ERG amplitudes (median: 0.41 units; interquartile range 0.34 –0.64; p=0.95) using the Mann–Whitney U nonparametric test (Figure 1). The results did not change when first degree relatives were excluded from the analysis (n=22): T:S ratios of 11 patients with highest cone ERG amplitudes (median: 0.39 units; interquartile range 0.36–0.56) were similar to the ratios of 11 patients with the lowest cone ERG amplitudes (median: 0.36 units; interquartile range 0.33 –0.64; p=0.38). Multiple regression analysis of the total group of 30 patients, adjusting for age and refractive error, also showed no significant relation between $\log_e$ 30 Hz-ERG amplitude and telomere T:S ratio (t=-0.75; p=0.46).

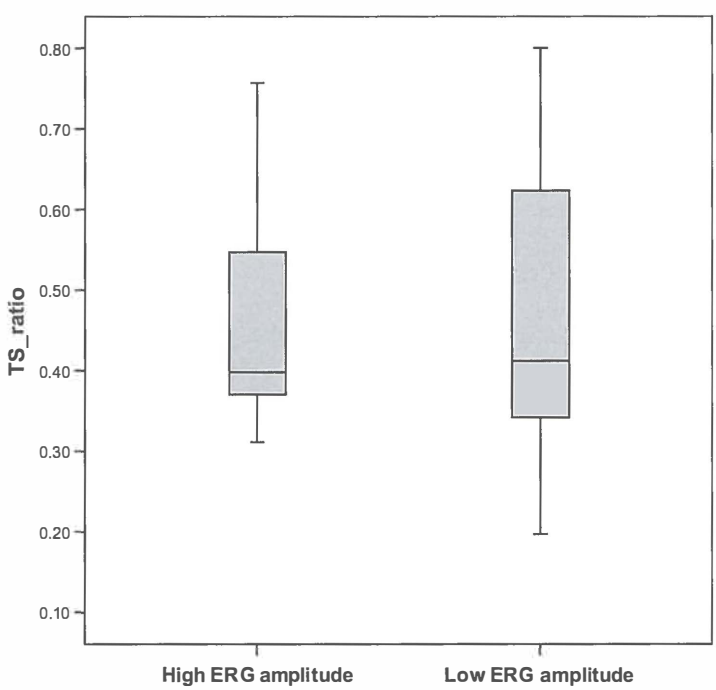


Figure 1 Relative telomere lengths in Pro23His RP patients. Boxplot showing no difference in T:S ratio's between patients with high- or low ERG amplitudes. The shaded boxes indicate the interquartile range. The horizontal line in each shaded box denotes the median, and the error bars mark the upper and lower 95 percentiles of the T:S ratio.

Discussion

The rhodopsin gene product is a transmembrane G-coupled protein (opsin). It is found in the rod outer segments, and, when bound with chromophore, mediates the initial steps of the phototransduction cascade.[36,37] The *RHO*-Pro23His mutation encodes a misfolded protein that aggregates within the endoplasmic reticulum[38,39] and seems to activate apoptosis by the unfolded protein response (UPR).[40-42] On average, patients with the *RHO*-Pro23His mutation tend to have milder disease compared to those with other rhodopsin mutations.[43-47] However, there is great variability in disease severity among those with Pro23His, and this variation can be objectively measured with ERGs.[48]

Since photoreceptors in RP appear to die ultimately through apoptosis, and since cells with chromosomes with short telomeres are prone to apoptosis, we hypothesized that patients with short telomeres might have more severe disease because their photoreceptors would more rapidly undergo apoptosis in response to the deleterious effects of *RHO*-Pro23His. To test this hypothesis, we confined our analysis to sets of patients at the extremes of disease severity as determined by 30-Hz cone ERG amplitudes. We found no evidence for an association between telomere length and severity of RP. However, a limitation of our analysis method must be noted: We used DNA derived from dividing leukocytes, since our cells of interest, the nondividing retinal photoreceptors, were not available from living patients. Although it is reported that telomere size is highly correlated among tissues,[49,50] it is known that dividing cells are subject to changes in telomere length with the main known factor being age. Since our groups of patients with mild and severe RP were of about the same ages, and since the effect of age is relatively small compared to the individual differences in telomere length, we assumed that the telomere length in the peripheral leucocytes reflected the telomere length in nondividing photoreceptor cells. However, a possible difference in telomere lengths between these two cell types cannot be ruled out. Our method using qRT–PCR measurements of telomere lengths has successfully been used in other studies and has been shown to correlate to the Southern blot method of telomere measurement.[51,52] However both methods provide only an estimate of actual telomere length.

References

1. Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. Lancet 2006; 368 (9549):1795-1809
2. Sheffield VC, Fishman GA, Beck JS, Kimura AE, Stone EM. Identification of novel rhodopsin mutations associated with retinitis pigmentosa by GC-clamped denaturing gradient gel electrophoresis. Am.J.Hum.Genet. 1991; 49 (4):699-706
3. Sung CH, Davenport CM, Hennessey JC, Maumenee IH, Jacobson SG, Heckenlively JR, Nowakowski R, Fishman G, Gouras P, Nathans J. Rhodopsin mutations in autosomal dominant retinitis pigmentosa. Proc.Natl.Acad.Sci.U.S.A 1991; 88 (15):6481-6485
4. Sohocki MM, Daiger SP, Bowne SJ, Rodriquez JA, Northrup H, Heckenlively JR, Birch DG, Mintz-Hittner H, Ruiz RS, Lewis RA, Saperstein DA, Sullivan LS. Prevalence of mutations causing retinitis pigmentosa and other inherited retinopathies. Hum.Mutat. 2001; 17 (1):42-51
5. Dryja TP, McGee TL, Reichel E, Hahn LB, Cowley GS, Yandell DW, Sandberg MA, Berson EL. A point mutation of the rhodopsin gene in one form of retinitis pigmentosa. Nature 1990; 343 (6256):364-366
6. Dryja TP, McEvoy JA, McGee TL, Berson EL. Novel rhodopsin mutations Gly114Val and Gln184Pro in dominant retinitis pigmentosa. Invest Ophthalmol.Vis.Sci. 2000; 41 (10):3124-3127
7. Berson EL, Rosner B, Sandberg MA, Dryja TP. Ocular findings in patients with autosomal dominant retinitis pigmentosa and a rhodopsin gene defect (Pro-23-His). Arch.Ophthalmol. 1991; 109 (1):92-101
8. To K, Adamian M, Dryja TP, Berson EL. Histopathologic study of variation in severity of retinitis pigmentosa due to the dominant rhodopsin mutation Pro23His. Am.J.Ophthalmol. 2002; 134 (2):290-293
9. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. Nature 1990; 345 (6274):458-460
10. Wong JM, Collins K. Telomere maintenance and disease. Lancet 2003; 362 (9388):983-988
11. McEachern MJ, Krauskopf A, Blackburn EH. Telomeres and their control. Annu.Rev.Genet. 2000; 34:331-358
12. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. Nature 1990; 345 (6274):458-460
13. Okuda K, Bardeguez A, Gardner JP, Rodriguez P, Ganesh V, Kimura M, Skurnick J, Awad G, Aviv A. Telomere length in the newborn. Pediatr.Res. 2002; 52 (3):377-381
14. Zhang X, Mar V, Zhou W, Harrington L, Robinson MO. Telomere shortening and apoptosis in telomerase-inhibited human tumor cells. Genes Dev. 1999; 13 (18):2388-2399
15. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. Nature 1990; 345 (6274):458-460
16. Greider CW. Telomere length regulation. Annu.Rev.Biochem. 1996; 65:337-365
17. Hastie ND, Dempster M, Dunlop MG, Thompson AM, Green DK, Allshire RC. Telomere reduction in human colorectal carcinoma and with ageing. Nature 1990; 346 (6287):866-868
18. Schwartz HS, Dahir GA, Butler MG. Telomere reduction in giant cell tumor of bone and with aging. Cancer Genet.Cytogenet. 1993; 71 (2):132-138
19. Vaziri H, Schachter F, Uchida I, Wei L, Zhu X, Effros R, Cohen D, Harley CB. Loss of telomeric DNA during aging of normal and trisomy 21 human lymphocytes. Am.J.Hum.Genet. 1993; 52 (4):661-667
20. Okuda K, Bardeguez A, Gardner JP, Rodriguez P, Ganesh V, Kimura M, Skurnick J, Awad G, Aviv A. Telomere length in the newborn. Pediatr.Res. 2002; 52 (3):377-381
21. Valdes AM, Andrew T, Gardner JP, Kimura M, Oelsner E, Cherkas LF, Aviv A, Spector TD. Obesity, cigarette smoking, and telomere length in women. Lancet 2005; 366 (9486):662-664

22. Youngren K, Jeanclos E, Aviv H, Kimura M, Stock J, Hanna M, Skurnick J, Bardeguez A, Aviv A. Synchrony in telomere length of the human fetus. Hum.Genet. 1998; 102 (6):640-643
23. Okuda K, Bardeguez A, Gardner JP, Rodriguez P, Ganesh V, Kimura M, Skurnick J, Awad G, Aviv A. Telomere length in the newborn. Pediatr.Res. 2002; 52 (3):377-381
24. Butler MG, Tilburt J, DeVries A, Muralidhar B, Aue G, Hedges L, Atkinson J, Schwartz H. Comparison of chromosome telomere integrity in multiple tissues from subjects at different ages. Cancer Genet.Cytogenet. 1998; 105 (2):138-144
25. van der Harst P, van der Steege G, de Boer RA, Voors AA, Hall AS, Mulder MJ, van Gilst WH, van Veldhuisen DJ. Telomere length of circulating leukocytes is decreased in patients with chronic heart failure. J.Am.Coll.Cardiol. 2007; 49 (13):1459-1464
26. Honig LS, Schupf N, Lee JH, Tang MX, Mayeux R. Shorter telomeres are associated with mortality in those with APOE epsilon4 and dementia. Ann.Neurol. 2006; 60 (2):181-187
27. van der Harst P, van der Steege G, de Boer RA, Voors AA, Hall AS, Mulder MJ, van Gilst WH, van Veldhuisen DJ. Telomere length of circulating leukocytes is decreased in patients with chronic heart failure. J.Am.Coll.Cardiol. 2007; 49 (13):1459-1464
28. Sandberg MA, Weigel-DiFranco C, Dryja TP, Berson EL. Clinical expression correlates with location of rhodopsin mutation in dominant retinitis pigmentosa. Invest Ophthalmol.Vis.Sci. 1995; 36 (9):1934-1942
29. Andreasson SO, Sandberg MA, Berson EL. Narrow-band filtering for monitoring low-amplitude cone electroretinograms in retinitis pigmentosa. Am.J.Ophthalmol. 1988; 105 (5):500-503
30. Berson EL, Rosner B, Sandberg MA, Hayes KC, Nicholson BW, Weigel-DiFranco C, Willett W. A randomized trial of vitamin A and vitamin E supplementation for retinitis pigmentosa. Arch.Ophthalmol. 1993; 111 (6):761-772
31. Cawthon RM. Telomere measurement by quantitative PCR. Nucleic Acids Res. 2002; 30 (10):e47
32. van der Harst P, van der Steege G, de Boer RA, Voors AA, Hall AS, Mulder MJ, van Gilst WH, van Veldhuisen DJ. Telomere length of circulating leukocytes is decreased in patients with chronic heart failure. J.Am.Coll.Cardiol. 2007; 49 (13):1459-1464
33. Epel ES, Blackburn EH, Lin J, Dhabhar FS, Adler NE, Morrow JD, Cawthon RM. Accelerated telomere shortening in response to life stress. Proc.Natl.Acad.Sci.U.S.A 2004; 101 (49):17312-17315
34. Grabowski P, Hultdin M, Karlsson K, Tobin G, Aleskog A, Thunberg U, Laurell A, Sundstrom C, Rosenquist R, Roos G. Telomere length as a prognostic parameter in chronic lymphocytic leukemia with special reference to VH gene mutation status. Blood 2005; 105 (12):4807-4812
35. Risch N, Merikangas K. The future of genetic studies of complex human diseases. Science 1996; 273 (5281):1516-1517
36. Hargrave PA. Rhodopsin structure, function, and topography the Friedenwald lecture. Invest Ophthalmol.Vis.Sci. 2001; 42 (1):3-9
37. Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, Le T, I, Teller DC, Okada T, Stenkamp RE, Yamamoto M, Miyano M. Crystal structure of rhodopsin: A G protein-coupled receptor. Science 2000; 289 (5480):739-745
38. Kaushal S, Khorana HG. Structure and function in rhodopsin. 7. Point mutations associated with autosomal dominant retinitis pigmentosa. Biochemistry 1994; 33 (20):6121-6128
39. Frederick JM, Krasnoperova NV, Hoffmann K, Church-Kopish J, Ruther K, Howes K, Lem J, Baehr W. Mutant rhodopsin transgene expression on a null background. Invest Ophthalmol. Vis.Sci. 2001; 42 (3):826-833
40. Frederick JM, Krasnoperova NV, Hoffmann K, Church-Kopish J, Ruther K, Howes K, Lem J, Baehr W. Mutant rhodopsin transgene expression on a null background. Invest Ophthalmol. Vis.Sci. 2001; 42 (3):826-833

41. Illing ME, Rajan RS, Bence NF, Kopito RR. A rhodopsin mutant linked to autosomal dominant retinitis pigmentosa is prone to aggregate and interacts with the ubiquitin proteasome system. J.Biol.Chem. 2002; 277 (37):34150-34160
42. Noorwez SM, Kuksa V, Imanishi Y, Zhu L, Filipek S, Palczewski K, Kaushal S. Pharmacological chaperone-mediated in vivo folding and stabilization of the P23H-opsin mutant associated with autosomal dominant retinitis pigmentosa. J.Biol.Chem. 2003; 278 (16):14442-14450
43. Sandberg MA, Weigel-DiFranco C, Dryja TP, Berson EL. Clinical expression correlates with location of rhodopsin mutation in dominant retinitis pigmentosa. Invest Ophthalmol.Vis.Sci. 1995; 36 (9):1934-1942
44. Oh KT, Weleber RG, Lotery A, Oh DM, Billingslea AM, Stone EM. Description of a new mutation in rhodopsin, Pro23Ala, and comparison with electroretinographic and clinical characteristics of the Pro23His mutation. Arch.Ophthalmol. 2000; 118 (9):1269-1276
45. Oh KT, Longmuir R, Oh DM, Stone EM, Kopp K, Brown J, Fishman GA, Sonkin P, Gehrs KM, Weleber RG. Comparison of the clinical expression of retinitis pigmentosa associated with rhodopsin mutations at codon 347 and codon 23. Am.J.Ophthalmol. 2003; 136 (2):306-313
46. Berson EL, Rosner B, Weigel-DiFranco C, Dryja TP, Sandberg MA. Disease progression in patients with dominant retinitis pigmentosa and rhodopsin mutations. Invest Ophthalmol. Vis.Sci. 2002; 43 (9):3027-3036
47. Berson EL, Rosner B, Sandberg MA, Weigel-DiFranco C, Dryja TP. Ocular findings in patients with autosomal dominant retinitis pigmentosa and rhodopsin, proline-347-leucine. Am.J.Ophthalmol. 1991; 111 (5):614-623
48. Berson EL, Rosner B, Sandberg MA, Dryja TP. Ocular findings in patients with autosomal dominant retinitis pigmentosa and a rhodopsin gene defect (Pro-23-His). Arch.Ophthalmol. 1991; 109 (1):92-101
49. Okuda K, Bardeguez A, Gardner JP, Rodriguez P, Ganesh V, Kimura M, Skurnick J, Awad G, Aviv A. Telomere length in the newborn. Pediatr.Res. 2002; 52 (3):377-381
50. Butler MG, Tilburt J, DeVries A, Muralidhar B, Aue G, Hedges L, Atkinson J, Schwartz H. Comparison of chromosome telomere integrity in multiple tissues from subjects at different ages. Cancer Genet.Cytogenet. 1998; 105 (2):138-144
51. Epel ES, Blackburn EH, Lin J, Dhabhar FS, Adler NE, Morrow JD, Cawthon RM. Accelerated telomere shortening in response to life stress. Proc.Natl.Acad.Sci.U.S.A 2004; 101 (49):17312-17315
52. Grabowski P, Hultdin M, Karlsson K, Tobin G, Aleskog A, Thunberg U, Laurell A, Sundstrom C, Rosenquist R, Roos G. Telomere length as a prognostic parameter in chronic lymphocytic leukemia with special reference to VH gene mutation status. Blood 2005; 105 (12):4807-4812

Chapter 4

Six Patients with Bradyopsia (Slow Vision): Clinical Features and Course of the Disease

Dyonne T. Hartong[1], Jan-Willem R. Pott[1] and Aart C. Kooijman[1,2]

[1]Department of Ophthalmology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands.
[2]Laboratory of Experimental Ophthalmology, School of Behavioural and Cognitive Neurosciences, University of Groningen, Groningen, Netherlands.

Ophthalmology. 2007 Dec;114(12):2323-31.

Abstract

Objective
Recently, it was discovered that subjects who showed a prolonged response suppression on their electroretinogram (ERG) and had symptoms of photophobia, problems adjusting to bright light, and difficulties seeing moving objects shared a mutation in the *RGS9* (regulator of G-protein signaling 9) gene that is involved in the deactivation of photoreceptor responses. The disorder was termed *bradyopsia* (slow vision). This paper reports the clinical presentation and long-term follow-up of 6 bradyopsia patients.

Design
Retrospective observational case series with a follow-up ranging from 6 to 30 years.

Participants
Six patients with a homozygous mutation in the *RGS9* gene.

Methods
Clinical symptoms and signs were compared between the subjects and between their visits over time.

Main Outcome Measures
Symptoms, visual acuity (VA), ocular findings, visual fields, dark-adaptation tests, color tests, fluorescein angiography, and ERG findings.

Results
Data showed a consistency in the individual symptoms and ERG recordings, but an extreme variation in VA between visits. Beside some irregularities in the macula in some patients, no other related eye abnormalities were seen. The low-to-subnormal VA varied with background luminance and typically increased by 2 to 3 lines when pinholes were used. Dark-adaptation tests, color tests, and fluorescein angiography were normal. Visual field tests showed a minor diffuse sensitivity loss. No progressive changes were seen over time.

Conclusions
No signs of progression were noted in the 6 bradyopsia patients. Photophobia, impaired movement perception, variable reduced VA that improved with the use of pinholes and ERG abnormalities were typical for the disease.

Introduction

Prolonged suppression of the electroretinal response (i.e., 5- to 10-fold longer than normal) was described in 4 Dutch patients in 1991.[1] The same prolonged electroretinal response suppression was noted a few years later in the electroretinograms (ERGs) of mice lacking the RGS9 protein.[2, 3, 4 and 5] In 2004, Nishiguchi et al[5] analyzed the *RGS9* and *R9AP* genes of the 4 Dutch patients and of 1 Guatemalan patient who had the same ERG characteristics. They discovered the homozygous missense mutation *W299R* (TGC to CGG; c.895T → C) in the *RGS9* gene of all 4 Dutch patients and a homozygous frameshift mutation R65 (1-base pair insertion; CGG to CCGG; c.194insC) in the *R9AP* gene of the Guatemalan patient. All of the patients had subnormal visual acuity (VA), complaints of photophobia, difficulties adjusting to changes in illumination, and problems seeing objects at ball games. Nishiguchi et al proposed the term *bradyopsia* (slow vision) for this new disease entity.[5]

In a normal phototransduction cascade, the transmembrane-bound rhodopsin molecule (in rods) is activated by a photon (light). This activation "turns on" a transducin molecule that, through activation of phosphodiesterase, decreases the amount of cyclic guanosine monophosphate available in the photoreceptor cell. This decrease results in a closure of the cyclic guanosine monophosphate-gated ion channels and a subsequent hyperpolarization of the photoreceptor outer segment membrane. This electrical current leads to the activation of downstream neuronal retinal cells. RGS9 (regulator of G-protein signaling 9) is a photoreceptor outer segment protein anchored to and stimulated by R9AP[6, 7 and 8] (regulator of G protein signaling 9-binding protein) that is involved in the deactivation of the turned on transducin molecule: It accelerates the rate of guanosine triphosphate hydrolysis by releasing phosphate.[9 and 10] This accelerative mechanism allows the rapid (in less than a second) return of the photoreceptor cell to its resting state. Transducin can return to its resting state without RGS9/R9AP, but the process takes tens of seconds.[11] During this time frame, the photoreceptor is temporarily immune to light activation. An individual with a defect in RGS9/R9AP, therefore, is blinded for a considerable amount of time after an initial light stimulus[9, 10, 11 and 12] and, as a result, has difficulties perceiving small dark structures against a bright background. This causes decreased VA or, after an initial bright flash like that used in ERG measurements, a response to subsequent flashes that is decreased for a longer period than in a normal eye.

All 4 Dutch bradyopsia patients were identified at the University Medical Center Groningen (Groningen, The Netherlands). When this paper was written, a total

of 6 patients at the hospital had been diagnosed as having the disorder. The first 5 subjects were identified between 1973 and 1993; the 6th was diagnosed recently. Four of the first 5 patients were reexamined in 2003. As a result, we had follow-up information spanning a 30-year period at our disposal. Based on our experience, the patients' symptoms and ocular findings can be rather confusing and may easily lead to an improper diagnosis. This paper addresses the problem and describes the disease phenotype associated with the *RGS9* mutation.

Materials and Methods

Six subjects with bradyopsia were studied (subjects 101, 102, 103, and 105 correspond to patient numbers 2, 1, 4, and 3, respectively, in Kooijman et al[1]; subjects 101, 103, 104, and 105 were also described by Nishiguchi et al[5]). The homozygous mutation *W299R* (TGC to CGG; c.895T → C) in the *RGS9* gene was confirmed as the cause of bradyopsia in all 6 subjects at the Ocular Molecular Genetics Institute (Boston, MA). Five of the 6 patients had had prior consultations at the Groningen hospital before the discovery of the gene mutation. We collected data from those visits from the patient records and from additional testing in 2003. The 6th patient was seen for the first time in 2004. The total number of visits to our clinic ranged from 2 to 7 (mean, 4.4) for subjects 101, 102, 103, 104, and 109; subject 105 was seen 15 times. Age at the last visit varied between 23 and 47 years. The group consisted of 4 females and 2 males. Table 1 presents the patients' characteristics. Two of the subjects (101 and 102) were directly related (brother and sister). Genealogical research revealed that 2 other subjects (103 and 109) were related consanguineously in the third degree (they had the same great-grandparents). One female subject mentioned having 2 brothers with the same complaints. They, however, did not want to participate in the study. Another female subject described a sister who had the same characteristics, but was unavailable for participation. Written informed consent was obtained from all subjects before participation in the study and the Medical Ethical Review Committee of the University Medical Center Groningen approved the study protocol. The study was consistent with the principles outlined in the Declaration of Helsinki.

Table 1. Basic Characteristics, Ocular Findings, and Results of Ocular Tests Other Than the Characteristic Electroretinogram (ERG) Recordings of 6 Bradyopsia Patients

Nr	Year of birth	Sex	Family	Refr OD	Refr OS	Typical VA	Increase with pinhole	Ocular findings	Visual field	Color vision	Dark adaptation	Other tests
101	1969	m	+ sister, subj.102	S +4.0 C -1.25	S +5.0 C -2.0	0.5 OD 0.08 OS	unknown	Nystagmus, esotropia and amblyopia OS; normal	MO 3-6 dB HFA diffuse sensitivity loss	Normal	Normal	OCT: normal Neurol. evaluat: normal
102	1964	f	+ brother, subj.101	S -0.5 C -1.5	S -1.5 C -1.5	0.5 OD 0.5 OS 0.7 ODS	3 lines	Somewhat irregularly pigmented maculae	Normal Goldmann	Non-specific errors	Normal	FAG: normal
103	1980	m	–	–	–	0.25 OD 0.25 OS	3 lines	Coarsely pigmented maculae/ normal	MO 5-6 dB HFA diffuse sensitivity loss	15 hue desat: non-specific errors	–	FAG: normal Neurol. evaluat: normal
104	1964	f	+ 2 brothers	S +1.5 C -1.5	S +1.0 C -1.75	0.5 OD 0.4 OS 0.7 ODS	2 lines	Minimal PE-alterations ODS	MO 2-2 dB HFA diffuse sensitivity loss	Normal	Normal	–
105	1980	f	–	S -0.5 C -1.25	C -1.25	0.6 OD 0.3 OS 0.7 ODS	2 lines	Chorioretinitis scar OS since 1990	–	Normal	–	VEP: normal EOG: normal EEG: normal
109	1957	f	+ sister	S -3 C -1.5	S -3.25	0.45 OD 0.4 OS 0.6 ODS	2-3 lines	IOL ODS; normal	MO 5-8 dB HFA diffuse sensitivity loss	Normal	Normal	Neurol. evaluat: normal

C = cylinder; dB = decibels; EEG = electro-encephalogram; EOG = electro-oculogram; F = female; FAG = fluorescence angiogram; HFA = Humphrey visual field analyzer; IOL = intraocular lens; M = male; MD = mean deviation; OCT = optical coherence tomography; OD = right eye; OS = left eye; PE = pigment epithelium; S = sphere; VA = visual acuity; VEP = visual evoked potential.

–, not evaluated.

Snellen VAs, assessed during earlier visits to our ophthalmology department or by a general ophthalmologist, were obtained from patient records. In 2003, the acuity scores were measured using Early Treatment Diabetic Retinopathy Study charts at luminances ranging from 0.1 to 1200 cd/m^2. Electroretinographic responses were measured using a Ganzfeld ERG lens with a built-in light-emitting diode stimulator. The scotopic and photopic stimulus conditions were the same as those in the International Society for Clinical Electrophysiology of Vision (ISCEV) standard protocol.[13 and 14] A double-flash protocol was used to assess the course of the response recovery. The pupils were dilated with eyedrops—1 drop of 0.5% tropicamide was used in 1991; a combination of 1 drop of 0.5% tropicamide and 1 drop of 2.5% phenylephrine was used in 2003. The eyes were dark adapted for ≥30 minutes before scotopic ERG measurements. According to the double-flash protocol, 2 standard flashes (0.8 log cd*s/m^2) were applied under the dark-adapted condition. The interval between the 2 flashes varied from 0.5 to 128 seconds and the interval between the last flash of 1 pair and the first flash of the next pair was always ≥120 seconds. Electroretinogram responses to stimuli, conformed to the ISCEV standard, were registered as the average of 10 to 20 responses. Double-flash responses were recorded as single responses. Either the Farnsworth D15-saturated and -desaturated or the Farnsworth-Munsell 100 Hue Test was used as the color vision test. Dark adaptation was assessed using the Goldmann-Weekers dark adaptometer, where the retina is initially bleached for 2 minutes and retinal responses are subsequently recorded every minute for the first 15 minutes, and then every 2 to 3 minutes for the second 15 minutes. Visual fields were documented with either the Goldmann Perimeter, the Octopus 500 (30°), or Humphrey Visual Field Analyzer (HFA; 30-2 threshold). Optical coherence tomography retinal mapping was performed in 1 patient using the Optical Coherence Tomography 2 (Zeiss-Humphrey Inc., San Leandro, CA).

Results

Subject 101, a male, was referred to our clinic by a general ophthalmologist in 1986 at the age of 17 years. He was known to have left esotropia and amblyopia, and nystagmus and hypermetropia in both eyes. His complaints consisted of photophobia and problems seeing at twilight. He also reported difficulties adapting from light to dark environments and vice versa. Seventeen years later at the age of 34 (in 2003), his symptoms had not changed. He said that he was unable to partake in any sports, because he was "not able to see it." His family history was positive with 1 older sister experiencing the same problems (subject 102 in this paper). Corrected VA (Snellen chart) at age 17 were measured as 0.5 in the right eye and 0.08 in the left eye (amblyopia). At age 34, his VA measurements varied 2 Snellen

lines when different background luminances were used (right eye; maximum 0.6 at 1–3 cd/m²; minimum 0.4 at 1200 cd/m²; Fig 1). No abnormalities were found during slit-lamp examination or fundoscopy. Dark-adaptation and color tests were normal at both 17 and 34 years of age. His visual fields (Octopus) at age 17 showed mild diffuse sensitivity loss (mean deviation/defect MD, −3.4 decibel dB right eye; −5.7 dB left eye) and a strongly decreased central sensitivity (−21 dB) in the amblyopic left eye. His visual field tests (measured with HFA) at age 34 showed similar results (MD, −3.22 dB right eye; −5.69 dB left eye). ERG testing was performed in 1986, 1987, 1988, and 2003 and showed rod responses with normal implicit times and a- and b-wave components to dim flashes in the dark adapted condition. The average mixed rod–cone response to the strong standard flash, however, showed a reduced b-wave amplitude (for an example, see Fig 2). No responses to single flashes or 30-Hz flashes were recorded under the light adapted condition. Remarkable was the much delayed recovery of the response to the second flash (between 16 and 32 seconds) in the dark-adapted condition during the double-flash measurements (Fig 3). The a- and b-wave components plus implicit time of the single flash response had not changed from the responses recorded 17 years earlier. Also, the a:b-wave ratio had not changed considerably (Fig 4, Table 2). Optical coherence tomography retinal mapping of the macular region was normal.

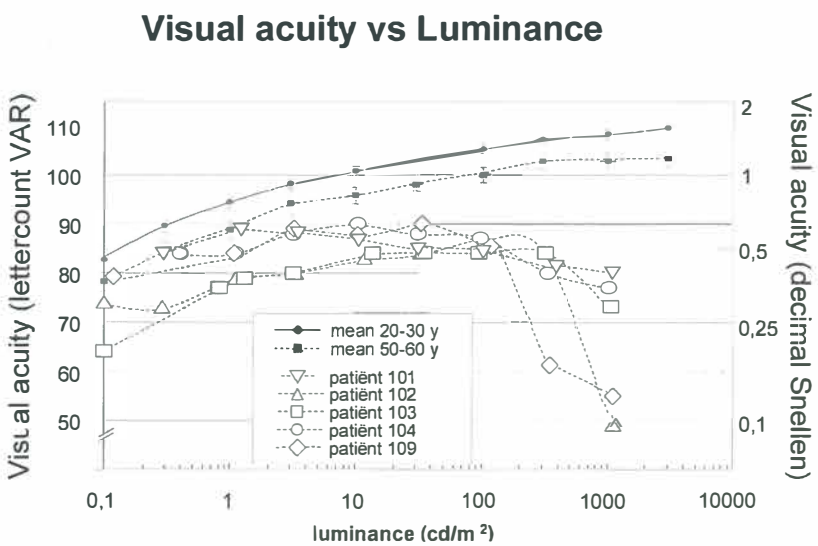


Figure 1. Chart showing decreasing visual acuities (right eye) in 5 bradyopsia patients when background luminance is increased. The upper 2 lines represent the change in visual acuity with changing background illumination in normal controls ages 20 to 30 years and normal controls ages 50 to 60 years. cd = candelas; VAR = visual acuity rating.

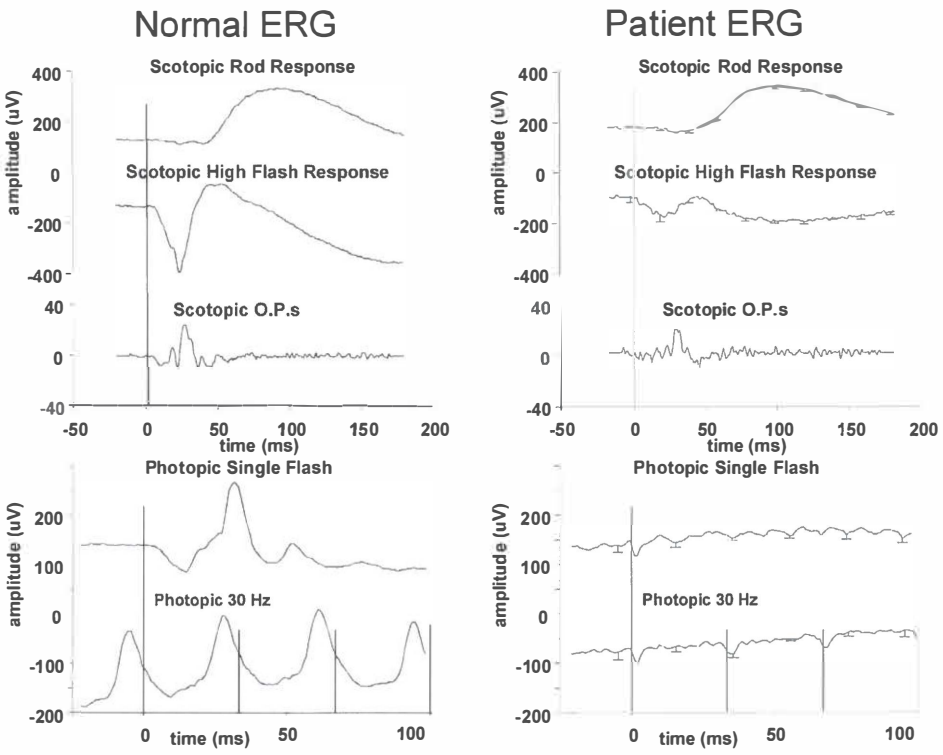


Figure 2. Routine electroretinograms (ERGs) showed normal responses to scotopic stimulus flashes in the bradyopsia patient. Responses to standard high-intensity flashes were markedly reduced in amplitude. Photopic responses were not recordable (subject 109). Hz = hertz; ms = milliseconds; O.P.s = oscillatory potentials.

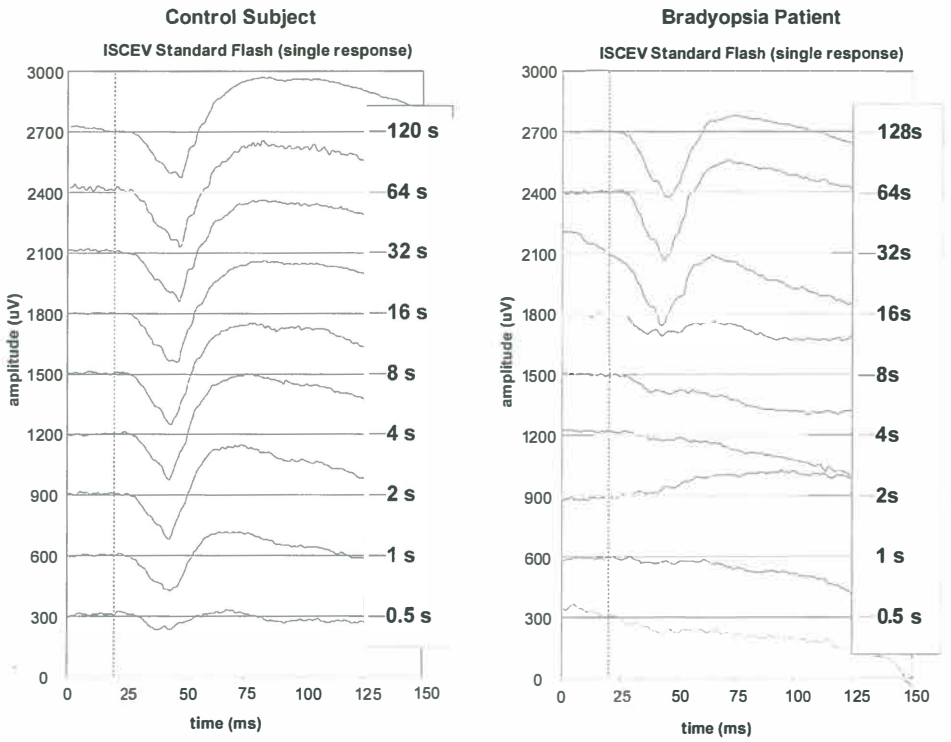


Figure 3. Left, Recovery of response after an initial standard flash occurs within 2 seconds in normal control subjects. **Right**, Recovery of response after an initial standard flash takes about 30 seconds in bradyopsia patients (subject 101). ISCEV = International Society for Clinical Electrophysiology of Vision.

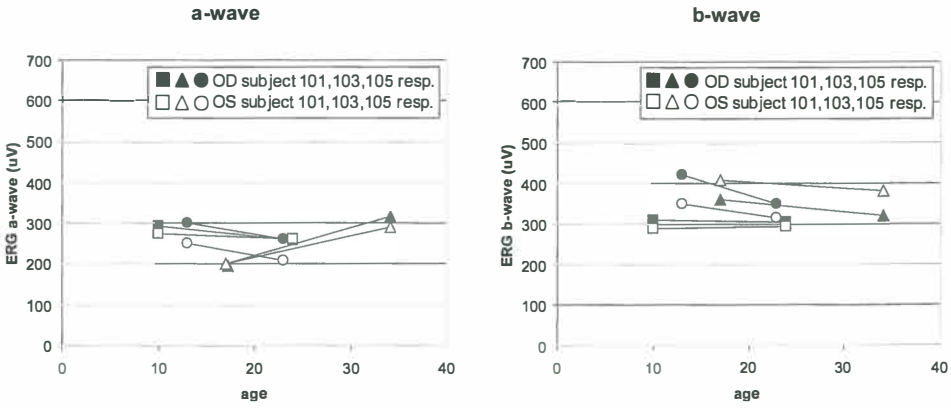


Figure 4. Figure illustrating mixed rod and cone electroretinogram (ERG) a-wave (**left**) and b-wave (**right**) responses for subjects 101, 103, and 104 at different ages. Single response to the standard flash (0.8 log candela*second/m^2) in the scotopic condition. Pretrigger time without any light stimulation: first visit, 30 seconds; second visit, 120 seconds. No evident changes in amplitudes were detected over the years. OD = right eye; OS = left eye.

Table 2. Comparison of Scotopic a- and b-Wave Amplitudes and the a:b-Wave Ratio for High-Intensity Flashes (Mixed Rod and Cone–Mediated Response) at Different Ages for 3 Subjects.

		Standard combined ERG 0.8 log cd*s/m², Scotopic Condition					
		Right eye			Left eye		
Subject	Year	a-Wave (uV)	b-Wave (uV)	a:b-Wave ratio	a-Wave (uV)	b-Wave (uV)	a:b-Wave ratio
101	1987	200	360	0.5	200	320	0.6
	2003	315	408	0.7	291	381	0.8
103	1990	292	311	0.9	274	306	0.8
	2004	258	290	0.9	262	296	0.9
104	1993	300	420	0.7	250	350	0.7
	2003	260	350	0.7	210	315	0.7

ERG = electroretinogram.

Subjects 101, 103, and 104 show comparable results over a period of 17, 13, and 10 years, respectively.

Subject 102, a female, is the older sister of subject 101. She was referred to our department by a pediatric neurologist at the age of 9 years in 1973. Her medical history consisted of an episode of meningoencephalitis 3 years earlier. She complained of extreme sensitivity to bright lights and blurred vision. At follow-up 30 years later (2003), she remarked that she had to wear sunglasses while driving. Swimming and playing ball games (tennis, beach volleyball) were impossible for her, but skiing was not a real problem if she wore proper sunglasses. As a child (9 years old), her Snellen VA was measured as 0.2 in her right eye and 0.1 in her left eye; her maculae were described as being somewhat irregularly pigmented. Further orthoptic and ophthalmoscopic evaluation revealed no abnormalities. Visual field tests (Goldmann) and fluorescein angiography were normal. Color testing only showed a few nonspecific errors. At that time, she was suspected of having a conversion syndrome. During an observational hospital stay, she finally managed to show normal vision (1.0) in both eyes with correction (sphere, −0.5 diopters D; cylinder, −1.5 D; axis, 10° in her right eye, and sphere, −2.0 D; cylinder, −1.5 D; axis, 170° in her left eye). During subsequent visits over a 3-year period, however, VAs of 0.5 were measured repeatedly in both eyes and could not be corrected with an eyeglass prescription. They did increase considerably (to 0.8) when pinholes were used. Her binocular VA was usually 0.7. During her 2003 visit (age 39), the subject's VAs changed from 0.5 in her right eye and in her left eye at low background luminances (100 and 33 cd/m²) to 0.1 in her right eye and 0.2 in her left eye at a higher background luminance (1100 cd/m²). An ERG was performed in 1988 (at age 24) because the subject's brother seemed to have a

strange ERG response at that time. Her ERG also revealed a prolonged adaptation time. The subject refrained from undergoing more vision tests in 2003.

Subject 103, a male, was first seen at the age of 2 years by a general ophthalmologist (1982); he seemed to have problems with fixation. At age 11 (1990), the boy reported photophobic complaints, but had no problems with vision in the dark. Twelve years later at age 23 (2003), he explained that he still had problems adapting to sunlight, but that his complaints were usually better after some time in the sun. Adaptation from light to dark circumstances was easier. The subject always wore sunglasses outdoors and while driving. He said that he played soccer, but was not able to see the ball while it was in flight. He was also easily blinded by the snow, but enjoyed skiing if he wore sunglasses. His VA was 0.9 in both eyes at the age of 3 and 4 years, 1.0 in both eyes at the age of 6 years, and only 0.1 in both eyes at the age of 10 years. The use of pinholes increased his VAs to 0.6 in his right eye and 0.7 in his left eye. One year later (at age 11), VAs of 0.25 were measured in both eyes, again showing improvement (0.5 in both eyes) when pinholes were used. During his 2003 visit (at age 23), the subject's VAs changed from 0.3 in his right and left eye at a high luminance (1069 cd/m^2) to a maximum of 0.5 at 13 to 315 cd/m^2 (right eye) and 0.6 at 13 cd/m^2 (left eye; Fig 1). Ophthalmoscopic and fundoscopic tests (performed in 1990) were normal except that his maculae were described as being coarsely pigmented. No retinal abnormalities were seen during the 2003 visit (Fig 5). At age 10, he performed the Farnsworth D15-desaturated hue test, which showed some nonspecific errors (left eye more than right eye). Repeated tests at age 23 (Farnsworth D15-saturated and -desaturated and Farnsworth-Munsell 100 hue), however, were completely normal. In 1990 (at age 10), Goldmann visual field testing revealed a slight central sensitivity loss in both eyes and a somewhat concentric visual field loss in the right eye. Humphrey visual field analyses during his visit in 2003 (age 23) showed a similar diffuse sensitivity loss (MD, −5.1 dB in his right eye; −5.8 dB in his left eye). His ERG measurements in 1990 were described as being normal to low-intensity stimuli and showing a prolonged response suppression (about 24 seconds) with higher intensity stimuli in the scotopic condition. Cone responses to stimuli under photopic conditions were absent. Initially, the subject was suspected of having Stargardt's disease. A fluorescein angiogram, however, showed no sign of the disorder. Later, the patient was suspected of having cone dystrophy, even though the improvement in his VA when pinholes were used could not be explained. In 2003 (at age 23), the subject was diagnosed with bradyopsia. At that time, a delayed recovery response ranging between 16 and 32 seconds was seen on the ERG. The a- and b-wave amplitudes and the a:b-wave ratio did not differ significantly from the measurements in 1990 (Fig 4, Table 2).

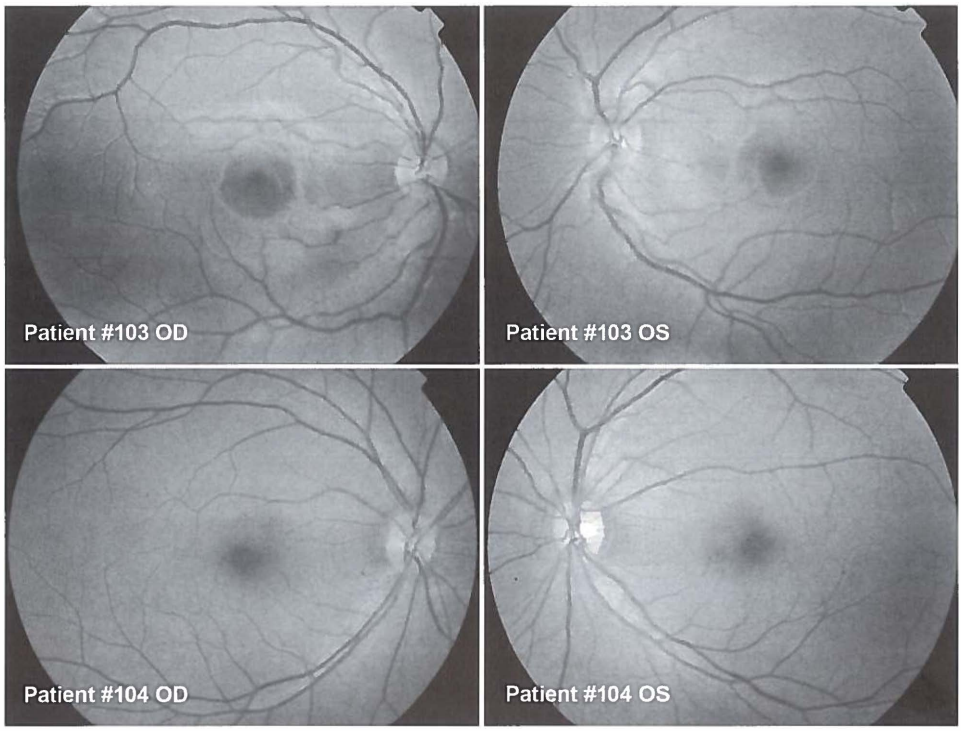


Figure 5. Figure illustrating normal fundus appearance in subject 103 and slight pigment alterations in the maculae of subject 104. OD = right eye; OS = left eye.

Subject 104, a female, was initially referred to our clinic for ERG measurements in 1993 at the age of 29. Her main complaint was photophobia. During her 2003 visit (age 39), she said that she always wore sunglasses while driving and that she was unable to see the ball while playing tennis, but could play badminton if she wore sunglasses. The subject also said that she could ski on sunny days if she wore appropriate sunglasses but that she was unable to ski on cloudy days because she could see no contrast. The subject seemed to have a positive family history for the symptoms: 2 brothers had the same complaints of photophobia. Visual acuities at her first visit (at age 29) were 0.5 (right eye) and 0.4 (left eye). In 2003 (at age 39), corrected VA (Snellen chart) were again measured as being 0.5 in her right eye (sphere, +1.25 D; cylinder, −1.5 D; axis, 150°) and 0.4 to 0.5 left eye (sphere, +1.0 D; cylinder, −1.75 D; axis, 21°), increasing to 0.7 in both eyes when pinholes were used. Her binocular acuity was also higher (0.7). Her VA measurements varied with changing background luminance: 0.35 (right eye) and 0.25 (left eye) at a high background luminance (1027 cd/m^2) and a maximum of 0.6 at lower background luminances (right eye, 3–32 cd/m^2; left eye, 10–32 cd/m^2; Fig 1). Slit-lamp examination revealed no abnormalities. Fundoscopy showed minimal

pigment-epithelium alterations in the fovea of the left eye (Fig 5). Dark-adaptation tests were normal with a final threshold of 1.5 log = $1.0*10^{-5}$ cd/m^2. Color tests were normal. Visual field testing using a Humphrey field analyzer showed diffuse sensitivity loss (MD, −2.0 dB, right eye; −2.3 dB, left eye) with normal foveal sensitivities. A delayed-response recovery was noted in her 1993 ERG measurements (≥24 seconds). The ERG response 10 years later (2003) showed similar a- and b-wave amplitudes and a:b-wave ratios (0.7 for both eyes; Fig 4, Table 2).

Subject 105, a female, visited our clinic in 1986 at the age of 6 years. At that time, she complained of photophobia that had existed for >1 year. Her mother remarked that the girl was not able to see the ball at ball games and that she could not avoid some obstacles while riding a bicycle in bright light. Her best-corrected VA at age 6 years was assessed to be 0.2 in both eyes. Six months later, her VAs had declined to 0.5/60 (right eye) and 0.1 (left eye). Reading at home, however, was not a problem. This was underlined by her ability to read the smallest reading text at the clinic. On follow-up visits, the girl's VAs varied greatly, up to a maximum of 0.9 Snellen acuity, and sometimes improved considerably when a pinhole was used. Ophthalmologic examination revealed no abnormalities. Her diagnosis remained unclear at the time. An ERG revealed lower response amplitudes at high-flash intensities under scotopic and photopic conditions, plus a prolonged recovery response time with subsequent flashes. It was concluded at this point that the patient had a retinal dysfunction, although the exact diagnosis was unclear. Subsequent electroencephalographic recordings and visual evoked potentials were normal. In 1990 (age 10), the patient developed a toxoplasmic chorioretinitis in the left eye. It was treated with co-trimoxazole and prednisolone, according protocol, but left a quiet scar superior to the fovea. In the years thereafter, the girl's complaints regarding adaptation to light remained. In 1992 and 1993, tests were repeated and additional tests were performed in another clinic. Her dark-adaptation tests were normal. Foveal densitometry revealed a decreased density of the visual (cone) pigment. Her electro-oculogram was normal. Her photopic ERG showed decreased a- and b-wave amplitudes with normal implicit times, and her scotopic ERG was on the lower edge of normal standards. Color-vision tests using an anomaloscope, the desaturated Farnsworth 15 Hue test, and Ishihara tests were all normal. The conclusion was a diagnosis of "oligocone trichromasia." In 2003, the patient agreed to donate blood for genetic evaluation. She did not want to undergo another ophthalmic evaluation.

Subject 109, a female, was seen for the first time in 2003 at the age of 47 years. Her history was consistent with those of the other 5 subjects: As long as she could remember, she had always had low VA and complaints of photophobia. She said

that she saw best when the light was dim. She wore sunglasses when she played indoor volleyball, but could not see the ball for a while when it was in front of a light source. She preferred to play badminton at twilight. She was taking driving lessons, but had to wear sunglasses. Her family history was positive; a sister had the same complaints of photophobia. Earlier that year, our subject underwent cataract surgery for a nuclear cataract in both eyes. Her preoperative best-corrected VAs were measured as 0.25 in her right eye (sphere, −3 D; cylinder, −1.5 D; axis, 175°) and 0.25 in her left eye (sphere, −3.25 D), increasing to 0.8 in both eyes when pinholes were used. Postoperative VAs only increased to 0.45 in her right eye and 0.4 in her left eye; binocular VA was 0.6. Snellen VAs tested at varying background luminances during the last visit showed values ranging from 0.12 (right eye) and 0.08 (left eye) at a luminance of 1040 cd/m^2 to 0.6 (in both eyes) at a luminance of 3 to 35 cd/m^2. Fundoscopic tests were normal. Initial visual field tests (HFA 10-2 threshold) showed paracentral scotomas in both eyes and decreased foveal sensitivities (−10 dB). Repeated HFA 30-2 testing the same year showed diffuse sensitivity loss (MD, −5.2 dB right eye; −8.3 dB left eye) with a great difficulty to fixate (tests had to be repeated because of too many fixation losses) and decreased foveal sensitivities (−8 dB right eye; (−11 dB left eye). Her scotopic a-wave was reduced in the initial ERG. No response was seen with photopic repeated stimulation. The patient was suspected of having cone dystrophy or rod monochromatism and referred to our clinic for further evaluation. Because "bradyopsia" had already been discovered, the subject was immediately suspected of suffering from it. Further tests revealed normal dark-adaptation tests and color tests (Farnsworth D-15). Scotopic ERG measurements conform to the ISCEV standards showed a typical bradyopsia pattern with normal implicit times at all flash intensities used, slightly decreasing a-wave amplitudes with increasing flash intensities, and a profound decrease in the b-wave amplitude of the averaged response to the standard flash stimuli delivered at 5-second intervals. No responses were present under standard ISCEV photopic conditions. The scotopic response to double-standard flash stimuli delivered at 16-second intervals showed decreased amplitudes. Only at 32-second intervals was the same amplitude measured as at 120-second intervals. DNA analysis confirmed the *RGS9* mutation.

Discussion

This compilation and long-term evaluation shows a clear consistency in the complaints and clinical findings of 6 patients diagnosed with bradyopsia and confirmed by a mutation in the *RGS9* gene. All subjects had photophobic complaints and noted having problems seeing moving objects, especially against a bright background (e.g., sky). The subjects also showed a considerable variation

in their VAs during subsequent follow-up visits. As shown by these case studies, this variation can easily lead to confusion or even misplaced conclusions of a patient's credibility. Most striking, but again confusing, was the increase in VA when pinholes were used; all efforts of refraction correction seemed to fail.

Based on the etiology of the disorder, all of the signs and symptoms now fall into place: After the photoreceptor is activated by an initial flash of light, the phototransduction cascade takes place and results in a hyperpolarized state of the photoreceptor cell. Only after deactivation of the members of the phototransduction cascade can the cell process subsequent light stimuli. Because the cell is insensitive during this time of recovery, the individual experiences a period of blindness. This period of insensitivity is so brief in normal individuals that they are not aware of the process. In patients lacking RGS9/R9AP (the molecules that accelerate the deactivation process of transducin a member of the phototransduction cascade), however, the photoreceptors are insensitive for a considerable period of time (tens of seconds). An increase in (background) light results in an increased level of activation of the photoreceptor cells and a decrease in the remaining dynamics of these cells, making them less sensitive to any subsequent light stimulus. As a result, a blinding effect and thus photophobia occurs with bright light. Moreover, the visibility of small structures, namely, the VA of these patients, decrease as the (background) light increases. Figure 1 shows the detrimental effect on the VAs of bradyopsia patients when background luminance increases >100 cd/m^2.

The blinding effect occurring with increasing background light also explains the variations in VAs seen at successive visits in the clinic. Changes in the 100- to 500-cd/m^2 luminance range have hardly any influence on the assessed VA in normal-sighted people. The luminance of the white background of acuity charts is usually in the 80- to 160-cd/m^2 range and that of the optotype projector image in the 100- to 700-cd/m^2 range. Study protocols specify a small tolerance with regard to the luminance of VA charts. This is, however, rarely addressed in regular clinical practice and can vary by a factor of 10. In bradyopsia patients, such variations in conditions can result in VAs ranging from near normal vision to severely decreased vision.

These individuals also complain of difficulties seeing moving objects, such as sports objects (e.g., a ball), against a background of extremely bright sky. This is understandable since the luminance of the sky can be as high as 10 000 cd/m^2,[15] resulting in a retinal illumination of 3 to 7 × 10^4 troland with a normal pupil diameter of 2 to 3 mm.[16] In normal human cones, a steady retinal illumination of 3 to 7 × 10^4 troland results in 50% to 75% of the cone pigment being bleached.[10]

The use of a pinhole diminishes the amount of light entering the eye and thus reduces the amount of light-induced stimulation of the photoreceptor cells. The use of sunglasses has the same effect on the light entering the eye. It is not surprising, therefore, that all of our subjects adapted their lifestyles to wearing sunglasses whenever they went outdoors during the day.

The ERG findings of a prolonged recovery time after an initial bright flash can also be explained by the prolonged activated state of the photoreceptor cell. Because the photoreceptor cells (the a-wave in an ERG recording) are the starting point of a retinal electrical evoked response, the secondary b-wave (inner retinal response) is affected as well; that is, no full signal is transferred from the photoreceptor to downstream retinal neuronal cells. In the resting state, the photoreceptor of a bradyopsia patient is normally sensitive to light stimuli and thus gives a normal initial response to a white flash. Because the time of the photoreceptor deactivation is prolonged, a longer time is needed between 2 flashes to generate a second normal sensitive response.

Our cases demonstrate that the anterior segment of the eye is not altered in patients with bradyopsia. Fundus examinations and optical coherence tomography retinal mapping did not indicate signs of retinal degeneration, although some inconsistent descriptions of minimal pigment alterations at the posterior pole have been reported. *RGS9* knockout mice showed morphologically normal retinas up to 8 months of age.[2] Rod and cone function was also not otherwise altered, as shown by normal dark adaptation (rod function), normal color tests (cone function), and the potential of near-normal VAs (cone function) under optimal (no bright light) conditions. Similar to the ERG, the dark-adaptation test is a measurement of responses to flashes of light. It is understandable that dark adaptation measured with the Goldmann-Weekers dark adaptometer was not affected by the slowed recovery response. The lowest luminance used in that test was 0.5×10^{-7} cd/m^2 and the luminance was increased in logarithmic steps until the patient saw the stimulus. The highest luminance of the stimulus was 0.5 cd/m^2. Such very dim light flashes activate only a small portion of the inactive transducin molecules in the photoreceptors, leaving enough inactive transducin molecules available for subsequent stimuli. Thus, the assessed dark-adaptation process was not influenced by the impaired deactivation of turned-on transducin molecules in the receptor.

Ten to 17 years of ERG follow-up measurements (Fig 4), 10 to 30 years of VA follow-up, and no degeneration of the patients' fundi all point to the stationary nature of the disease. The time of ERG recovery after a bright stimulus flash

remained unchanged and the a- and b-wave amplitudes and the a:b-wave ratio of the scotopic single-flash responses did not differ significantly between the old and new measurements. Visual acuity measurements, in contrast, differed significantly during follow-up measurements, but did not show a general decline over the years. This can be attributed to the nonstandardized lighting conditions during subsequent acuity assessments. It must, however, be stated that this study comprised a rather small sample size. A larger sample size with a structured follow-up should provide more significant proof of the stationary state of the disease.

The differential diagnosis of the main complaint of photophobia is quite extensive, ranging from temporary corneal problems to retinal diseases such as achromatopsia. Electroretinogram recordings conform to a dedicated protocol, along with other distinctive signs, however, provide the distinction between bradyopsia and other causes of the symptom. Although the ERGs assessed conform to the standard ISCEV protocol and showed similarities with recordings from cone-rod dystrophy or typical achromatopsia patients with a loss of scotopic b-wave amplitude at higher flash intensities, reduced or absent photopic amplitudes, and loss of cone-flicker responses, the prolonged recovery response, demonstrated by double-flash electroretinography, is not seen in these disorders. Congenital achromatic patients are classified as having typical (rod) monochromatism or atypical (cone) monochromatism.[17] The complete type of typical (rod) monochromatism has symptoms of decreased VA, poor color vision, photophobia, and nystagmus. The incomplete type of typical (rod) monochromatism is a less pronounced variant of the complete type. Atypical (cone) monochromatism will not be confused with bradyopsia because of atypical monochromatism shows a normal photopic response and severely disturbed scotopic responses. Cone–rod dystrophies may also cause decreased VA, nystagmus, photophobia, and decreased color vision.[17] These patients, however, also eventually show visual field defects and fundus abnormalities, such as retinal vessel attenuation, pigment clumping, and macular lesions. A simple essential initial differential tool to test for bradyopsia is the pinhole test. It shows an improvement in VA (after adequate refraction and the exclusion of cataract) that is not seen in other retinal dystrophies. Another differential test is the color-vision test: It was normal or only slightly aspecifically altered in our bradyopsia patients, but is abnormal in patients with the other disorders. We also want to emphasize the importance of evaluating the entire set of recordings with every regular ERG interpretation instead of only reading the automatically averaged wave amplitudes. The typical bradyopsia pattern of a normal first-flash amplitude and smaller subsequent flash recordings may easily be missed in the latter case.

In summary, bradyopsia patients usually present in childhood with a specific history of photophobic complaints, difficulties seeing moving objects on a bright background, and problems adjusting to light changes. They usually have subnormal VAs that improve with the use of pinholes, but not or only partially with refraction correction. No other obvious eye abnormalities are observed. Color and dark-adaptation tests are normal; visual field tests show no field defect, but often indicate a general reduced central sensitivity. Averaged single-flash scotopic ERG measurements typically show reduced amplitudes with higher flash intensities, whereas averaged single flash responses to dim light (rod response) are normal in amplitude. The time to generate a fully recovered response to the second strong flash after a normal response to the first strong flash is severely prolonged when paired flash tests are used. The diagnosis of bradyopsia can be made on the basis of ERG measurements and confirmed by the detection of a mutation in either the *RGS9* or *R9GS* gene. There were no signs of a progression of the disorder in our sample group. When needed, advice for adjustments in daily life or simple visual aids that reduce brightness should be given.

References

1. Kooijman AC, Houtman A, Damhof A, van Engelen JP. Prolonged electro-retinal response suppression (PERRS) in patients with stationary subnormal visual acuity and photophobia. Doc Ophthalmol. 1991;78:245-254.
2. Chen CK, Burns ME, He W, Wensel TG, Baylor DA, Simon MI. Slowed recovery of rod photoresponse in mice lacking the GTPase accelerating protein RGS9-1. Nature. 2000;403:557-560.
3. Lyubarsky AL, Naarendorp F, Zhang X, Wensel T, Simon MI, Pugh EN, Jr. RGS9-1 is required for normal inactivation of mouse cone phototransduction. Mol Vis. 2001;7:71-78.
4. Martemyanov KA, Lishko PV, Calero N, et al. The DEP domain determines subcellular targeting of the GTPase activating protein RGS9 in vivo. J Neurosci. 2003;23:10175-10181.
5. Nishiguchi KM, Sandberg MA, Kooijman AC, et al. Defects in RGS9 or its anchor protein R9AP in patients with slow photoreceptor deactivation. Nature. 2004;427:75-78.
6. Baker SA, Martemyanov KA, Shavkunov AS, Arshavsky VY. Kinetic mechanism of RGS9-1 potentiation by R9AP. Biochemistry. 2006;45:10690-10697.
7. Hu G, Zhang Z, Wensel TG. Activation of RGS9-1GTPase acceleration by its membrane anchor, R9AP. J Biol Chem. 2003;278:14550-14554.
8. Hu G, Wensel TG. Characterization of R9AP, a membrane anchor for the photoreceptor GTPase-accelerating protein, RGS9-1. Methods Enzymol. 2004;390:178-196.
9. He W, Cowan CW, Wensel TG. RGS9, a GTPase accelerator for phototransduction. Neuron. 1998;20:95-102.
10. He W, Wensel TG. RGS function in visual signal transduction. Methods Enzymol. 2002;344:724-740.
11. Blumer KJ. Vision: the need for speed. Nature. 2004;427:20-21.
12. Krispel CM, Chen D, Melling N, et al. RGS expression rate limits recovery of rod photoresponses. Neuron. 2006;51:409-416.
13. Kooijman AC, Damhof A. A portable ERG system with an automatic driven LED Ganzfeld stimulation contact lens. Ophthalmologica. 1981;182:224-228.
14. Marmor MF, Fishman GA. At last. A standard electroretinography protocol. Arch Ophthalmol. 1989;107:813-814.
15. Lee RL, Jr., Hernandez-Andres J. Short-term variability of overcast brightness. Appl Opt. 2005;44:5704-5711.
16. Wyszecki G, Stiles WS. Color science, 2nd ed. New York: John Wiley & Sons; 1982.
17. Fishman GA. The electroretinogram in retinal disease. In: Fishman GA, Sokol S, Electrophysiologic testing in disorders of the retina, optic nerve, and visual pathway. American Academy of Ophthalmology, 1990; 1-90.

Chapter 5

Improved Mobility and Independence of Night-Blind People Using Night-Vision Goggles

Dyonne T. Hartong,[1,2] Frank F. Jorritsma,[3] Johannes J. Neve,[4]
Bart J. M. Melis-Dankers,[5] and Aart C. Kooijman[1,2,5]

[1]From the Laboratory of Experimental Ophthalmology, School of Behavioral and Cognitive Neurosciences, University of Groningen, The Netherlands;
[2]Department of Ophthalmology, University Hospital Groningen, Groningen, The Netherlands;
[3]Vision Rehabilitation Center, Bartimeus Utrecht, The Netherlands; the [4]Vision Rehabilitation Center, Sensis Eindhoven, The Netherlands; and the [5]Vision Rehabilitation Center, Visio Loo Erf, Huizen, The Netherlands.

Invest Ophthalmol Vis Sci. 2004 Jun;45(6):1725-31.

Abstract

Purpose

To investigate whether the use of night-vision goggles (NVGs) by night-blind people improves their mobility and sense of independence under dark circumstances.

Methods

Twenty night-blind subjects with retinitis pigmentosa were requested to walk predetermined routes at night with and without NVGs. The number of unintended contacts with obstacles (hits) and the percentage of preferred walking speed (PPWS) en route were assessed in three different situations: a darkened indoor corridor; a moderately lit outdoor residential area; and a well-lit outdoor shopping area. Assessments were performed before and after a 5-week training period, during which the subjects practiced using NVGs in their own surroundings, registered their experiences in a journal, and filled out questionnaires.

Results

The mean number of hits in the darkened corridor declined from eight to two when NVGs were used. Mean PPWS (34%) did not improve. In the residential area, mean hits declined from eight to practically zero and mean PPWS increased from 60% to 72% (after training to 78%). In the shopping area, subjects walked at 93% PPWS without any hits and showed no improvement with NVGs. Subjective scores revealed a good sense of orientation, feelings of safety and tranquility and an increase in independent mobility when NVGs were used.

Conclusions

Using NVGs seems to improve nighttime mobility in dark outdoor conditions by decreasing unintended contacts with obstacles and increasing walking speed. Use of NVGs increased independent activities in these subjects and was generally positively evaluated for everyday outdoor use.

Introduction

Night-blindness is caused by an impaired rod function of the outer sensory retina and is a symptom of a number of inherited retinal disorders. The congenital form, congenital stationary night blindness (CSNB) is not progressive and has no other accompanying disturbed visual functions. Retinitis pigmentosa (RP), the best-known type of retinal degeneration, is progressive and involves both rods and cones. Because damage to the rod system usually predominates in the early stages of the disease, the first symptom of RP is often night blindness. It is followed by an increasing loss of peripheral visual field and deterioration of visual acuity in later stages. Night-blind subjects perceive the outdoor environment after sunset as almost completely dark. They bump into objects, their orientation is usually seriously hindered, and their walking speed is substantially reduced.[1 2 3] In addition, independent travel and other outdoor activities often become impossible. Night blindness therefore severely interferes with normal daily activities.

A luminance-enhancing vision aid may be of great help to night-blind individuals. In the past, light-enhancement devices were large, heavy monocular instruments,[4] that later evolved into smaller,[5] more compact, hand-held devices.[6] These were then followed by head-mounted binocular instruments, known as night-vision goggles (NVGs). Today, a newer version of these spectacles, called the Multi-Vision (Trivisio, Taegerwilen, Switzerland), is available (Fig. 1) . It has the added advantage of a higher resolution and an improved automatic light-adapting system. Two studies investigating the potential benefit of NVGs were performed recently.[7 8] The one by Friedburg et al.[7] found that night-blind subjects could improve their visual functions in a laboratory design using NVGs. The second study[8] was conducted in a real-life situation, in which participants subjectively evaluated the device after walking a designated route. It reported that a small majority of participants was positive about the instrument. Although both studies provided new information, they did not show data about the influence of such a device on orientation and mobility in a realistic outdoor situation and they were both based on single experiences. To evaluate the usefulness of such a device adequately, we believe that more data should be assessed from real-life outdoor situations: not only from a single use, but also after a period with ample opportunity to practice using the instrument.

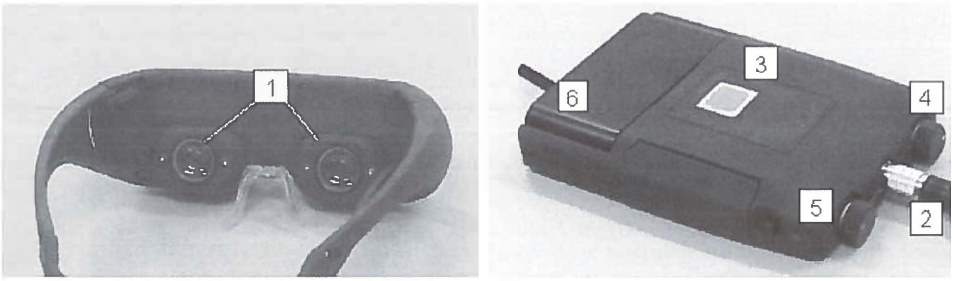


Figure 1. NVGs with attached power unit: (1) liquid crystal video (LCD) screens, (2) connecting cable, (3) push-button for power, (4) brightness control, (5) contrast control, and (6) rechargeable battery.

The purpose of the present study was to assess how much night-blind individuals benefit from using NVGs in everyday life. Data were collected on mobility performance (walking speed and the number of times obstacles were hit, referred to as hits) and personal judgments after prolonged use of a night-vision device.

Methods

Subjects

Night-blind subjects who wished to improve their nighttime mobility were recruited by means of announcements and advertisements at visual rehabilitation centers and the Dutch Retina Association. Walking cane users were initially excluded, because we wanted to test the function of NVGs alone. Because the visual field of the goggles used in this study was only 30°, we mainly addressed subjects with RP. Most of these individuals already had constricted visual fields and therefore were not expected to experience any severe additional field loss. No control group was included. We considered the test group as its own control group, since the subjects all performed tests both with and without NVGs. Pretests on visual acuity, visual field, and dark adaptation were conducted at the regional vision rehabilitation centers. Our study group (n = 20) consisted of 4 (20%) women and 16 (80%) men. All subjects had RP. Age distribution, visual acuity, visual field, and light sensitivities are presented in Table 1 . One subject was an active cane user, but did not use his cane during the time of the research. Written informed consent was obtained from all subjects before the study and the Institutional Review Board of the University Hospital of Groningen approved the study protocol. The study was consistent with the principles outlined in the Declaration of Helsinki.

Table 1. Characteristics of 20 Night-Blind Subjects with Retinitis Pigmentosa

Variable	Minimum	Maximum	Mean ± SD
Age	23	61	47.6 ± 11.52
Visual acuity OU* LogMAR	0.09 -1.05	1.0 0.00	0.53 ± 0.26 - 0.35 ± 0.29
Visual Field OU* (Goldmann III/4ᵉ-diameter) Visual Field Score†	6° 10	45° 62	21.65 ± 10.46 39.95 ± 14.53
Elevation of dark adaptation threshold (l.u.) compared to mean normal values (Goldmann Weekers adaptometer)	1.6	>5.0	3.1 ± 0.97

* OU, oculus unitas (both eyes).

†Visual field score according to Colenbrander et al.[14] is designed to indicate the severity of consequences of field defects. It involves a count score of 100 points distributed on a visual field grid; 50 points are confined to the central 10° of the visual field and another 50 points are confined to the periphery up to 60°. The points are located along 10 meridians, two in each of the upper quadrants, three in each of the lower quadrants.

Night-Vision Goggles

Twenty Multi-Vision night-vision devices (Trivisio; Fig. 1), were available for the duration of the study. (Fifteen of the devices were borrowed from Trivisio and five were owned by the research laboratory.) The Multi-Vision system consists of goggles (122 g, 155 x 50 x 50 mm) connected to a power unit (380 g, including battery, 155 x 105 x 25 mm), a microcamera (sensitivity 0.015 lux) located at the center of the goggles that records images of the visual world (horizontal diameter 30°), and a connecting cable that transfers the signals to the power unit for signal processing. The black-and-white image is presented at an enhanced luminance level within the goggles to both eyes on two super video graphics array (SVGA) displays with 480,000-pixel color resolution (equals 1,440,000 pixels). Contrast and brightness can be manually adjusted on the power unit. If ambient light is insufficient, additional illumination can be achieved by switching on two built-in infrared-light sources. Pupil distance and nose position can be altered to align the images with both eyes. The accompanying rechargeable battery enables an operating time of up to 2 hours (~1.5 hours when the infrared light is switched on).

Routes

The two indoor laboratory walking routes consisted of a darkened corridor with floor-level illumination (at +20 cm) between 10^{-3} and 10^{-2} lux, and a route length of 36 meters. Ten artificial rectangular obstacles were placed along the route at

different heights (foot, knee, shoulder, and head). The four outdoor walking routes (>0.5 hour after sunset) in the residential area each covered 330 meters and had floor-level illumination between 10^{-2} and 10^{-1} lux. The four routes at the outdoor shopping areas were 187 meters long and had illumination levels between 10^{-1} and 10 lux. The outdoor routes had comparable amounts of obstacles: curbs, public gardens, lampposts, and poles, for example.

Test Protocol

All subjects were consecutively invited to our laboratory in Groningen, twice during the dark winter season of 2002 to 2003. The first visit started with instructions on the use of the Multi-Vision. Then, the individual preferred walking speeds (PWSs) were assessed by measuring the walking speed of each participant three times along an unobstructed, straight path (17 m).[9] This was followed by orientation and mobility tests first without and second with use of NVGs along the three dark walking routes (indoor corridor, outdoor residential area, and shopping area). Every test route differed and thus was unknown to the participant. An initial walking route with NVGs without scoring was performed before starting the first test. Furthermore, the order of routes randomly changed between subjects. During the test routes, subjects had to follow a predetermined route, of which we had measured the exact distance. To follow the route, subjects were instructed by the investigator to turn either to the right or left at the next crossing. This information was given long before a particular junction was reached, so the subjects had to find their own way.

All the subjects were lent a Multi-Vision to use during the4 to 6 weeks between visits. They were requested to practice using the device in their own surroundings every evening and to register their experiences in a journal. The subjects also received weekly mobility instructions and feedback from a professional mobility trainer. After this training period, the subjects returned to Groningen for their second visit. There, the orientation and mobility tests were repeated and personal experiences discussed. All subjects were requested to fill out a questionnaire regarding their nighttime walking experiences at start and after the training period.

Scores: Objective

Orientation and mobility performance along the routes was scored as hits and percentage of preferred walking speed (PPWS). Hits were scored as the number of unintended contacts with obstacles along the walking route (i.e., curbs, poles, garden fences, and public gardens). This score reflects the level of "risk" on a route.[10] PWS assumes that subjects walk at their optimal walking speed when they do not have to worry about obstacles or dangerous conditions en route. Walking speed (meters per second) on a test route is calculated as the percentage of the preferred

walking speed (PPWS) and is a measure of "walking efficiency."[11] The number of unintended contacts with obstacles and the time to cover a route were recorded by the investigator. All data were collected and converted on computer (SPSS for Windows; SPSS Science, Chicago, IL).

Scores: Subjective

Questionnaires consist of 22 questions considering "specific problems with mobility," "bumping into obstacles," and "independent travel." The experienced trouble on these items was scored with help of a 5-point Likert scale: 1, never; 2, sometimes; 3, regular; 4, often; and 5, always. Research by Turano et al.[12] has shown that a similar questionnaire is a valid way to measure perceived ability for independent mobility in persons with RP. However, this questionnaire has not been validated for the particular nighttime condition. Therefore, we will confine our results to a descriptive evaluation of median scores.

The journal consists of judgments about difficulty, recognition, orientation, and feelings of safety and tension after every daily walking-route with use of NVGs. Values on these items were scored by use of an appreciation scale form 1 (very low appreciation) to 10 (very high appreciation).

Statistics

The significance of the differences in PPWS and hits with and without NVGs was tested using the nonparametric Wilcoxon test at the 0.01 level. The relation between the ophthalmic features and the results was tested at the 0.01 level with the Spearman's correlation test. Visual acuity was expressed as logarithm of the minimum angle of resolution (logMAR)[13] for the analyses. Visual fields were calculated as the visual field score according to Colenbrander.[14] (The visual field score replaces the Esterman grids and is proposed by Colenbrander,[14] accepted by the International Council of Ophthalmology (http://www.icoph.org/pdf/visualstandardsreport.pdf), and included in the fifth edition of the AMA *Guides to the Evaluation of Permanent Impairments*.[15] See also Table 1.)

Results

Data on mean hit scores are presented in Figure 2 . When NVGs were used, the mean number of hits decreased from eight to two ($P < 0.001$) in the indoor corridor and from eight to practically zero ($P < 0.001$) on the residential routes. Results of these hit scores did not differ between the first and second visits. No hits occurred in the shopping area, whether or not NVGs were used.

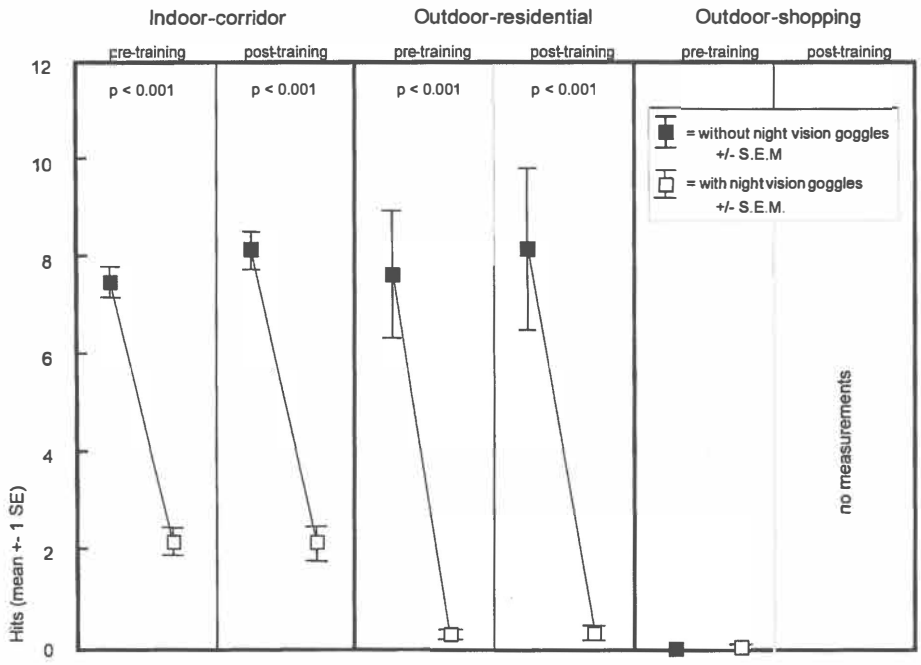


Figure 2. Number of obstacles hit. Indoor corridor: the mean of approximately eight hits decreased to two hits when NVGs were used. Outdoor residential area: mean of approximately eight hits decreased to almost zero when the goggles were used. Results at pre- and posttraining visits did not differ. Outdoor shopping area: practically no hits occurred without or with the use of the goggles.

Data on PPWS are illustrated in Figure 3. The mean PPWS was 1.48 m/s. Mean PPWS along the indoor corridor was 34% (0.51 m/s). This did not improve with use of NVGs at either the pre- or the posttraining visit. The PPWS along the residential routes was 60% (0.87 m/s), increasing to 72% (1.06 m/s; $P = 0.001$) when NVGs were used during the first visit, and 78% (1.16 m/s; $P < 0.001$) after training. PPWS without NVGs did not differ between the first and second visit. The mean rise in PPWS with use of NVGs after training was significant ($P = 0.005$). The PPWS at the shopping area was 93% (1.37 m/s), and slightly less (88%, 1.30 m/s) when the goggles were used.

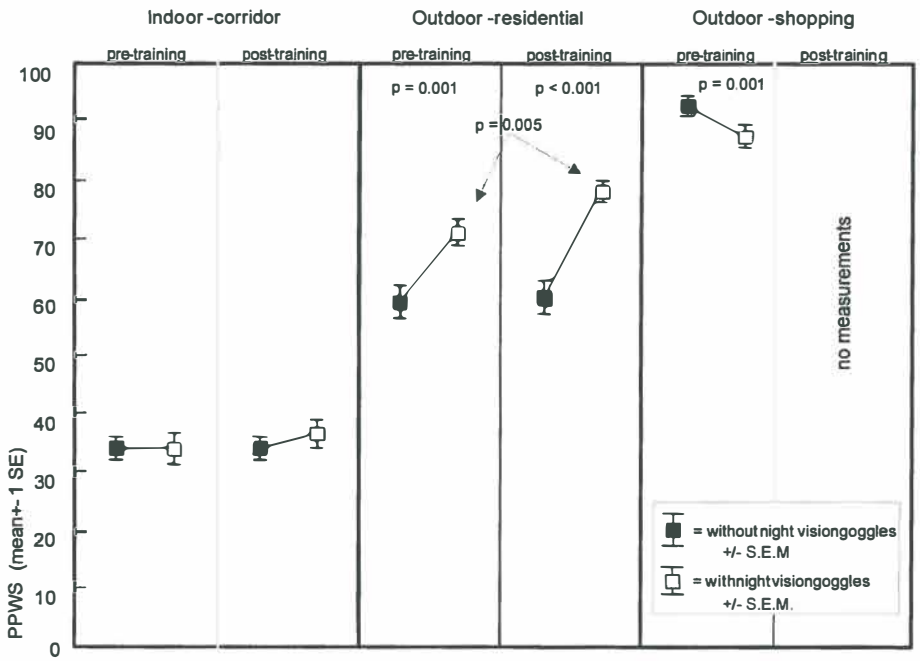


Figure 3. Percentage of PPWS. Indoor corridor: mean PPWS of 34% did not improve when NVGs were used. Outdoor residential area: mean PPWS of 60% increased to 72% when NVGs were used the first time and to 78% after training. Outdoor shopping area: mean PPWS of 93% decreased to 88% when the goggles were used.

The walking speed without NVGs in the shopping area at first visit was approximately normal and there were no hits on obstacles, which indicates that there was no impaired mobility due to vision problems. Because the goal of the study was to test the NVGs in situations in which vision is impaired, we decided not to perform tests in the shopping area during the second visit.

The increase in walking speed (r = 0.572) and for a great part the hit-score (r = 0.54) was related to the dark-adaptation thresholds but not to age, sex, visual acuity (logMAR), or visual field score (Table 2).

Table 2. Relationship between the Results and Sex, Age, Visual Acuity, Visual Field, and Dark-Adaptation Threshold

	Number of hits without NVG	Decrease in hits with NVG	PPWS without NVG	Increase in PPWS with NVG
Sex	- 0.130	- 0.120	- 0.260	- 0.260
Age	- 0.077	- 0.095	0.090	- 0.432
LogMAR	- 0.384	- 0.421	0.099	- 0.483*
Visual Field Score	- 0.331	- 0.289	0.440	0.073
Dark-adaptation threshold	0.549*	0.535*	- 0.207	0.572**

Comparisons are by Spearsman's nonparametric correlation test.
*p<0.05
**p<0.01

The subjective scores of data in the journals revealed a good sense of orientation, recognition, and feelings of safety and tranquillity during mobility when the goggles were used. The scores increased until the third week of use (mean scores >9 on a scale of 1 to 10; Fig. 4) and then remained at that level.

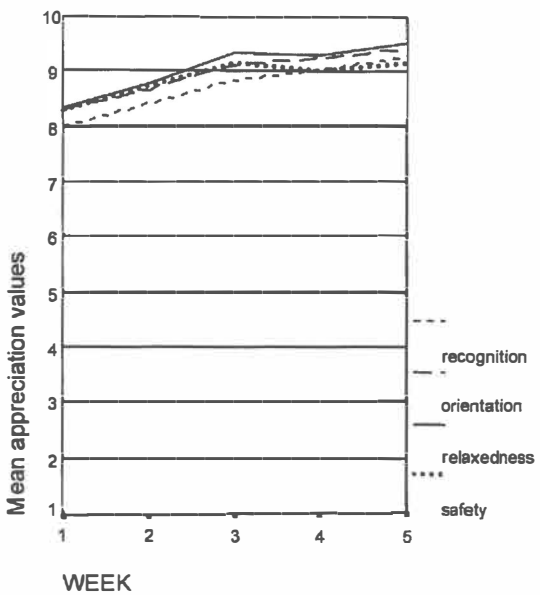


Figure 4. Mean scores per week as indicated in the subjects' journals for recognition, orientation, and feelings of tranquility and safety, while walking in the dark and using NVGs. Subjective appreciation of all four aspects ranged between 8 and 8.5 from the start of the training period and increased further until week 3 to more than 9. The scale is from 1 (low appreciation) to 10 (highest appreciation).

Responses to the questionnaires also showed fewer problems with nighttime walking, changes in light conditions, and bumping into obstacles when the NVGs were used (Fig. 5). In addition, the subjects noted that they traveled independently more often at night from the time when they started wearing the goggles (change in response was from "traveling always with guidance" to "traveling sometimes with guidance"). Difficulties that were experienced using the Multi-Vision system were noted as "glittering light sources", "problems with distance estimation", and "a constricted field of view". The subjects indicated that adaptation was established within a period of 2 to 3 weeks. At the second visit, 17 (85%) of the subjects were positive and 3 (15%) negative about using the Multi-Vision.

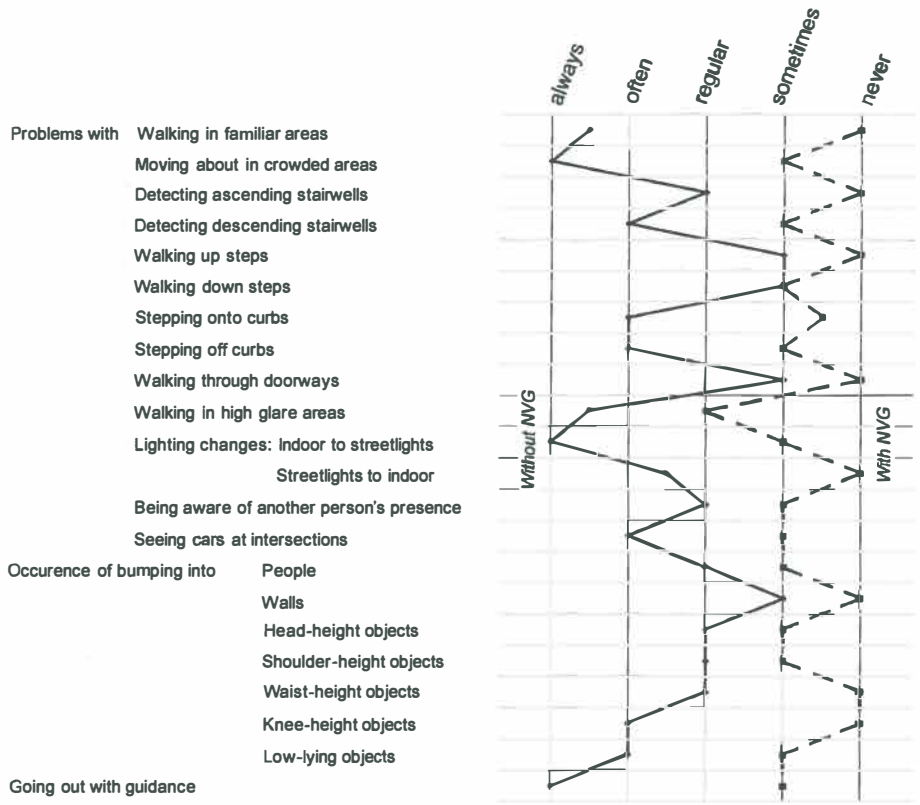


Figure 5. Median of answers to questionnaires regarding nighttime mobility without (*solid line*) and with (*dashed line*) NVGs.

Discussion

In accordance with earlier studies,[1, 2, 3] we found that many night-blind individuals bumped into obstacles while they walked unaided in the dark. Not using vision aids is likely to increase the risk of incidents with resultant morbidity.[16 17 18 19 20] As expected, in our study night-blind subjects indicated a very low frequency of independent activities or travel under nighttime conditions. NVGs, developed for use by night-blind people, have been available for some time. However, very little research has been performed to assess their practical value. To provide more extensive information on NVGs with regard to mobility and a sense of independence, we collected both objective and subjective data from night-blind people after a prolonged use of the instrument under realistic outdoor conditions.

Using NVGs on the outdoor residential test route clearly improved orientation and mobility and was expressed as a decrease in hits and an increase in walking speed.

Results were already highly significant at the first visit and therefore were independent of mobility training. Most striking was the immediate change in obstacle avoidance by every subject, resulting in practically no contacts with curbs, poles, fences, or other obstacles. Use of the goggles therefore is considered to improve safety while walking, since the risk of injuries or accidental falls caused by hits is likely to be reduced. At the second visit, walking speed and hit score without use of NVGs had not changed. However, with the use of NVGs, the general walking speed improved further after just a few weeks of practice, indicating an additional positive effect of training on mobility with NVGs.

At the start of the study, we did not know at which level of artificial streetlight vision would be sufficient for night-blind people. Our test showed the street-lighting levels at the shopping street (between 10^{-1} and 10 lux) were strong enough for "normal" mobility (no hits on obstacles and a normal walking speed). Also, our results showed no benefit from NVGs in this particular condition, which is in line with a recent evaluation study on NVGs by Bowers et al. (Bowers AR, et al. *IOVS* 2003,44:E-Abstract 2772).

The indoor corridor was the darkest of the three test conditions, and the walking speed there was extremely low. Because there was no improvement in walking speed when the goggles were used, the low walking speed was probably mostly due to the numerous artificial obstacles placed over the short distance. Binocular depth perception is not possible with the Multi-Vision, since both eyes receive an image from the same camera. This seemed to cause people to walk slowly in anticipation

of reaching an object. This problem with distance estimation together with the intensive scanning needed to detect the obstacles, randomly placed at head and feet height, also is probably the reason that the hit score did not reach zero in the indoor corridor as it did in the outdoor environment. Furthermore, its field of view is rather small, which at the short distances existing under indoor conditions, limits the opportunities to anticipate obstacles along the route. We presume that an overview is achieved easier on outdoor streets with larger distances. In other words, the instrument is considered to be less effective under indoor conditions.

For the measurements in all conditions it should be noted the without NVGs route always proceeded the with NVGs route, which may have biased our test results in favor of the goggles. It would have been more correct if we had changed these conditions. Yet, we believe a great consequence from a learning effect is implausible, because all performed test routes were different and thus new to the participant. Also, an initial walking route without scoring with NVGs was performed before starting the first test, which is considered as the primary practice route. Furthermore, at the second visit, walking speed and hit score without use of NVGs had not changed, indicating no effect from learning.

The mainly positive subjective evaluations given by night-blind people after several weeks of intensive use imply that the instrument is not only effective, but is also appreciated in practical use. The questionnaires and journals also revealed more independent travel during the dark evening hours. This does not, however, mean that there is no room for improvement. The most pronounced problem with the Multi-Vision involved experiences with light sources (e.g., car headlights, lit shop windows), which were perceived as unpleasant sparkling light spots within an otherwise intact view. Another frequently reported difficulty was the fact that no depth perception could be experienced using the instrument. Participants reported that they became accustomed to the two-dimensional view in many situations after several weeks of practice, but that this definitely did not apply to situations in which they had to estimate the distance to approaching cars. Some subjects also mentioned difficulties with the restricted visual field. Although these individuals already had constricted fields due to their disease, with use of NVGs, the perceived view can be increased only by head movement. Without the goggles, eye movements could accomplish this increase easier and faster. During mobility training, the participants were trained to enlarge their visual field by scanning the environment with systematic movements of the head. Experiences and the successful application of this scanning method, however, differed between the subjects. To increase comfort and facilitate utilization, future improvements to NVGs should include development of a better automatic light-

adapting system, the implementation of binocular vision, and the enlargement of the visual field.

Our study was designed primarily to indicate the potential effectiveness of NVGs. As we were restricted by the number of devices available and the limited period with dark evenings in the winter season, we could include only 20 participants. We selected participants with constricted central visual fields, because the study by Rohrschneider et al.[8] showed a better outcome within this group. We cannot, therefore, make any statements regarding the potential benefits for night-blind people with normal visual fields (e.g., those with congenital stationary night blindness) or for people with impaired central visual fields.

This study found no relationship between the results (objective and subjective) and visual acuity, visual field, sex, or age. The only relationship found was that subjects with more impaired light-sensitivities had more hits while walking unaided and, therefore, showed a larger reduction in the number of hits plus a higher increase in walking speed when using the goggles. The subjective improvement in independent mobility, however, did not differ between people with different levels of impairment.

Other available night-vision aids are the white cane and the wide-beam flashlight.[6] A comparison between NVGs and these instruments can be interesting and might be a subject for future research. From our study results, we consider NVGs as an alternative vision aid that has also been proven effective.

In our opinion, a potential NVGs candidate is a night-blind subject who declares him- or herself unable to move safely and independently under dark conditions. These individuals should be given the opportunity to practice using the instrument for 2 to 3 weeks to assess individual benefit.

In conclusion, at dark, outdoor conditions in which night-blind subjects have been shown to have considerable mobility problems, the NVGs seemed to improve mobility by decreasing hitting of obstacles and increasing walking speed. Use of the goggles increased independent nighttime activities in our subjects and was generally positively evaluated for everyday outdoor use. In very well-lit outdoor environments as exists in shopping streets, luminance is sufficient for normal mobility, and NVGs were of no additional value. The instrument may be less effective in indoor environments in terms of gain in walking speed, though it decreased the number of hits on obstacles considerably.

References

1. Geruschat DR, Turano KA, Stahl JW. Traditional measures of mobility performance and retinitis pigmentosa. *Optom Vis Sci.* 1998;75:525–537.
2. Kuyk T, Elliott JL, Fuhr PS. Visual correlates of mobility in real world settings in older adults with low vision. *Optom Vis Sci.* 1998;75:538–547.
3. Black A, Lovie-Kitchin JE, Woods RL. Mobility performance in retinitis pigmentosa. *Clin Exp Optom.* 1997;80:1–12.
4. Berson EL, Mehaffey L, III, Rabin AR. A night vision device as an aid for patients with retinitis pigmentosa. *Arch Ophthalmol.* 1973;90:112–116.
5. Berson EL, Rabin AR, Mehaffey L, III. Advances in night vision technology: a pocketscope for patients with retinitis pigmentosa. *Arch Ophthalmol.* 1973;90:427–431.
6. Morrisette DL, Marmor MF, Goodrich GL. An evaluation of night vision mobility aids. *Ophthalmology.* 1983;90:1226–1230.
7. Friedburg C, Sery L, Sharp LT, Trauzettel-Klosinski S, Zrenner E. Evaluation of night vision spectacles on patients with impaired night vision. *Graves Arch Clin Exp Ophthalmol.* 1999;237:125–136.
8. Rohrschneider K, Spandau U, Wechsler S, Blankenagel A. Utilization of a new night vision enhancement device (DAVIS) (in German). *Klin Monatsbl Augenheikd.* 2000;217:88–93.
9. Soong GP, Lovie-Kitchin JE, Brown B. Preferred walking speed for assessment of mobility performance: sighted guide versus non-sighted guide techniques. *Clin Exp Optom.* 2000;83:279–282.
10. Marron JA, Bailey IL. Visual factors and orientation-mobility performance. *Am J Optom Physiol Opt.* 1982;59:413–426.
11. Clark-Carter DD, Heyes AD, Howarth CI. The efficiency and walking speed of visually impaired people. *Ergonomics.* 1986;29:779–789.
12. Turano KA, Geruschat DR, Stahl JW. Perceived visual ability for independent mobility in persons with retinitis pigmentosa. *Invest Ophthalmol Vis Sci.* 1999;40:865–877.
13. Bailey IL, Lovie JE. New design principles for visual acuity letter charts. *Am J Optom Physiol Opt.* 1976;53:740–745.
14. Colenbrander A. The functional vision score. Kooijman AC Looijestijn PL Welling JA van der Wildt GJ eds. *Low Vision: Research and New Developments in Rehabilitation.* 1994;552–561. IOS Press Amsterdam.
15. *Guides to the Evaluation of Permanent Impairment.* 2000; 5th ed. American Medical Association Chicago.
16. Ivers RQ, Cumming RG, Mitchell P. Visual impairment and falls in older adults: the Blue Mountains Eye Study. *J Am Geriatr Soc.* 1998;46:58–64.
17. Klein BE, Klein R, Lee KE. Performance-based and self-assessed measures of visual function as related to history of falls, hip fractures, and measured gait time The Beaver Dam Eye Study. *Ophthalmology.* 1998;105:160–164.
18. Ivers RQ, Norton R, Cumming RG. Visual impairment and risk of hip fracture. *Am J Epidemiol.* 2000;152:633–639.
19. Ivers RQ, Cumming RG, Mitchell P. Visual risk factors for hip fracture in older people. *J Am Geriatr Soc.* 2003;51:356–363.
20. Klein BE, Moss SE, Klein R. Associations of visual function with physical outcomes and limitations 5 years later in an older population: the Beaver Dam eye study. *Ophthalmology.* 2003;110:644–650.

PIGMENT

Chapter 6

Night-vision goggles for night-blind subjects: subjective evaluation after 2 years of use

D. T. Hartong [1,2] and A. C. Kooijman [1,2]

[1] Laboratory of Experimental Ophthalmology, School of Behavioural and Cognitive Neurosciences, University of Groningen, Groningen , and [2] Department of Ophthalmology, University Medical Center Groningen, University of Groningen

Ophthalmic Physiol Opt. 2006 Sep;26(5):490-6.

Abstract

Purpose
To evaluate the usefulness of night-vision goggles (NVG) for night-blind subjects after 1 and 2 years of use.

Methods
Eleven night-blind subjects with retinitis pigmentosa used NVG for a 2-year period. At the end of each year, they were requested to fill-in two questionnaires regarding their use of the instrument.

Results
At both the 1- and 2-year evaluations, the subjects reported fewer problems with mobility in the dark when they used the goggles. At the 2-year evaluation, two individuals stated that they had stopped using the instrument, while seven used it at least twice a week. The nine subjects still using the instrument after 2 years reported an increase in their sense of independence and an increase in their potential for activities. The instrument was used most often during the dark winter season and for purposes such as visiting friends and family, travelling to work or sports, attending meetings and strolling.

Conclusion
At the 2-year follow-up, NVG were still being used by most of the night-blind subjects. Moreover, the device had a positive effect on the subjects' experienced opportunities and sense of independence. The instrument was considered useful in the daily lives of our subjects.

Introduction

Night blindness is one of the major symptoms of a number of inherited retinal disorders. Night blindness may be present as part of a progressive degenerative retinal process like retinitis pigmentosa (RP) or can also present as a stationary congenital disorder (e.g. congenital stationary night blindness). Night-blind subjects experience seriously impaired orientation and mobility under reduced lighting conditions (Black et al., 1997; Geruschat et al., 1998; Kuyk et al., 1998). The results of our previous study (Hartong et al., 2004) also support these findings as we found that 20 RP subjects regularly bumped into obstacles and also had very slow walking speeds along dark outdoor residential test routes. These individuals indicated in a questionnaire that they almost never went out alone when it was dark.

As night blindness often starts at a very young age, the disorder can be a limiting factor in the lives of these individuals. Except in the case of RP where Vitamin A therapy and omega-3-rich fish diets have been reported to slow the progression of RP (Berson et al., 1993, 2004), no treatments are available to cure these diseases. Physicians and rehabilitation specialists should, however, be able to suggest useful visual aid options. Unfortunately, there is very little literature available to date on aids for night blindness which may be useful in everyday life. Morrissette et al. (1983) compared mobility performance of night-blind subjects on outdoor test routes using either no vision aid, a wide-field lantern or a monocular light-enhancement device. Improvement in mobility (less bumps with obstacles and a higher walking speed) was shown with the use of the wide-field lantern but not with the use of the monocular light-enhancement device. Friedburg et al. (1999) found a benefit from night-vision spectacles in both contrast and motion perception for night-blind subjects in an indoor test setting. Rohrschneider et al. (2000) and Spandau et al. (2002) tested an early version of binocular night-vision goggles (NVG) for night-blind patients on an outdoor test route. The mobility trainer observed an improved mobility and orientation in 61% of the patients. Most of these subjects also subjectively experienced a beneficial effect from this device while walking the route. The findings of these studies, however, were based on just a single use of the instruments, and it is unclear whether or not similar results would be found with long-term use.

Night-vision goggles are used as a head-mounted instrument. This electronic device has a built-in camera that records an image of the surrounding environment. The image is light-enhanced and presented in real-time to the user on two black-and-white displays located in front of the eyes. In our previous study (Hartong et al.,

2004), we tested the use of a new NVG by night-blind RP patients on dark indoor and outdoor (residential area and well-lit shopping area) walking routes. Mobility performance was compared on routes with and without the use of NVG. Results indicated an improvement in the mobility performance for all subjects on the dark outdoor residential test routes. This finding was confirmed by a more recent study by Bowers et al. (2004), in which NVG were compared with a prototype Minified Augmented-View device.

To adequately evaluate the usefulness of a vision aid, it is also necessary to assess performance with the aid over an extended period of time. At the conclusion of our previous study (Hartong et al., 2004), 11 of the RP participants bought NVG. We contacted them after 1 and 2 years of use and compiled data on their experiences by means of questionnaires. This paper is the first evaluation of the long-term use of NVG by night-blind subjects.

Methods

Eleven (10 males and 1 female) of the 20 (16 males and 4 females) night-blind subjects with RP from the Hartong et al. (2004) study bought NVG ('Multi-Vision'; Trivisio, Taegerwilen, Switzerland) at the end of the study period. The subjects agreed to be contacted again for additional questionnaires regarding their use of the instrument. After 1 and 2 years (during the winter on both occasions), the subjects received two questionnaires which they were requested to fill-in and return with the use of an enclosed return envelope. If a response was delayed, the subject was called and encouraged to complete and return the questionnaires. Although all 11 subjects completed the questionnaires after the first year, 2 of them had stopped using the instrument during the second year and therefore did not fill-in the second set of questionnaires. Age, gender, visual acuity, visual field and dark-adapted thresholds of the individual subjects are given in Table 1. Visual fields were concentrically constricted in all patients because of their disease. Patients had been selected on this item in the initial study, so that they would not experience an (severe) additional field loss because of the constricted visual field (30°) of the NVG. Written informed consent was obtained from each individual before the study, and the Institutional Review Board of the University Hospital of Groningen approved the study protocol. The study was consistent with the principles outlined in the Declaration of Helsinki.

Table 1. Characteristics of the 11 night-blind subjects with retinitis pigmentosa.

Subject	Sex	Age	Diagnosis	Visual Acuity [Metric (Feet)] OD	Visual Acuity [Metric (Feet)] OS	Visual Acuity [Metric (Feet)] OU	Elevation of Dark Adapted Threshold [log units]	Visual Field Diameter [degree] OD	Visual Field Diameter [degree] OS
1	M	34	RP	0.67 (20/30)	0.67 (20/30)	0.8 (20/25)	3.1	40	30
2	M	47	RP	0.5 (20/40)	0.13 (20/150)	0.5 (20/40)	4.2	22	30
3	M	57	RP	0.4 (20/50)	0.16 (20/125)	0.4 (20/50)	1.8	20	20
4	M	31	RP	0.25 (20/80)	0.29 (20/70)	0.29 (20/70)	3.3	38	30
5	M	39	RP			0.59 (20/34)	3.5	8	10
6	F	56	RP	0.08 (20/250)	0.12 (20/170)	0.14 (20/140)	2.5	23	24
7	M	51	RP	0.56 (20/36)	0.059 (20/34)	0.8 (20/25)	1.9	19	15
8	M	61	RP	0.08 (20/250)	0.15 (20/130)	0.15 (20/130)	5.0	16	16
9	M	50	RP	0.09 (20/220)	0.06 (20/330)	0.09 (20/220)	5.0		11
10	M	31	RP	0.4 (20/50)	0.05 (20/400)	0.5 (20/40)	3.4	31	37
11	M	25	RP	0.5 (20/40)	0.5 (20/40)	0.5 (20/40)	2.3	19	15

The first questionnaire (questionnaire I-3, at 1 year and I-4, at 2 years) measured the perceived visual ability for independent mobility in persons with RP (Turano et al., 1999). It was the same questionnaire that the participants filled-in during the Hartong et al. (2004) study before they started using NVG (I-1) and again after using them for 6 weeks (I-2). It consisted of 22 questions about 'specific problems with mobility', 'bumping into obstacles' and 'independent activities'. The frequency with which specific problems were experienced by the participants was scored on a 5-point Likert scale: 1 – never; 2 – sometimes; 3 – regularly; 4 – often; and 5 – always. Median answers on the questionnaires I-1, I-2, I-3 and I-4 were compared by means of a descriptive evaluation, and significance was tested using a Wilcoxon signed rank test.

The first part of the second questionnaire (questionnaire II) consisted of eight multiple-choice questions on satisfaction and independence with the use of NVG. The specific questions with answer possibilities, and the individual responses are listed in Table 2. The remaining questions consisted of a 5-point Likert scale: 1 – never; 2 – sometimes; 3 – regularly; 4 – often; and 5 – always, and considered the use (period, frequency and purpose), and limitations of the goggles which had been identified in our previous study: lack of depth perception, restricted visual field, image disturbances, incorrect fitting of the frame and glaring of light sources. Evaluation consisted of a description of the median responses from 11 completed questionnaires at year 1 and 9 completed questionnaires at year 2.

Table 2. Multiple-choice questions of the second questionnaire (II) with answers of 11 subjects at the first year (black squares ■) and 9 subjects at the second year (open circles ○) (subjects 3 and 5 had discontinued the use of night-vision goggles (NVG) by the second year)

Subject		1	2	3	4	5	6	7	8	9	10	11
Are you satisfied with your purchase of NVG?	Very	◘	◘		◘	■	◘	■	■		◘	■
	Reasonably			■				○	○	◘		○
	No opinion											
	No											
Are you satisfied with the use of NVG?	Very	■	■		◘	■	◘	◘			◘	
	Reasonably	○	○	■					◘	◘		◘
	No opinion											
	No											
My opportunitues to perform activities since the use of NVG are	Much increased		◘		◘		◘			■	◘	○
	Partly increased	◘		■		■		◘	◘	○		■
	No change											
	Decreased											
My sense of independence since the use of NVG is	Much increased	○	◘		◘		◘			■	◘	◘
	Partly increased	■		■		■		○	◘	○		
	No change							■				
	Decreased											
I go out in the dark more often since the use of NVG.	Completely agree	○	◘		◘		○	○				
	Mainly agree	■					■	■	◘	■	◘	■
	No change					■				○		○
	Mainly disagree			■								
	Completely disagree											
I go out in the dark **alone** more often since the use of NVG.	Completely agree	○	◘		○		■	◘	■		○	
	Mainly agree	■		■	■		○		○	◘	■	○
	No change					■						■
	Mainly disagree											
	Completely disagree											
Do you use the NVG in a familiar or unfamiliar environment?	Only familiar					■			○			
	Mostly familiar, sometimes unfamiliar		◘		◘		◘	◘	■	◘	◘	◘
	Equally familiar/ unfamiliar	◘										
	Mostly infamiliar, sometimes familiar											
	Always unfamiliar											
How often do you use the NVG?	Daily		■		◘		■				■	○
	2-4 times a week	○	○			■	○			◘	○	
	About once a week	■						■	■			
	Less than once a week			■				○	○			■
	Never											

Results

Mobility

In our previous study (Hartong et al., 2004), questionnaires I-1 and I-2 showed that our subjects experienced fewer problems with mobility in the dark, with changes in light circumstances, and with bumping into obstacles after a 6-week use of NVG compared with the period without the goggles. These individuals also indicated that they participated in independent activities more often since they started using the goggles. This study, after 1 and 2 years of use of the goggles (questionnaires I-3 and I-4), again showed a statistically significant decrease of their reported mobility problems compared with the period without the use of NVG, with p values <0.01 for all individuals. Figure 1 indicates the median of reported mobility problems by the nine subjects who still used the device after 2 years. The use of NVG resulted in a three-level improvement in 'going out with guidance'. A two-level mobility improvement is reported for 'walking in familiar areas', 'moving about in crowded areas', 'detecting descending stairwells', 'stepping onto/off curbs', 'light changes from indoor to streetlights' and 'bumping into waist-height and knee-height objects'. Furthermore, a one-level improvement is seen with 'detecting stairwells and steps', 'walking through doorways', 'seeing cars at intersections' and 'bumping into people, walls or head-height objects'. The goggles did not change having problems with 'walking down steps', 'the awareness of another persons presence' and the 'bumping into shoulder-height objects'. These items were, however, only 'sometimes' experienced as a problem. Subjects did 'regularly' report having problems with 'moving about in crowded areas' and with high-glare sources (e.g. headlights from cars, streetlights) when using NVG.

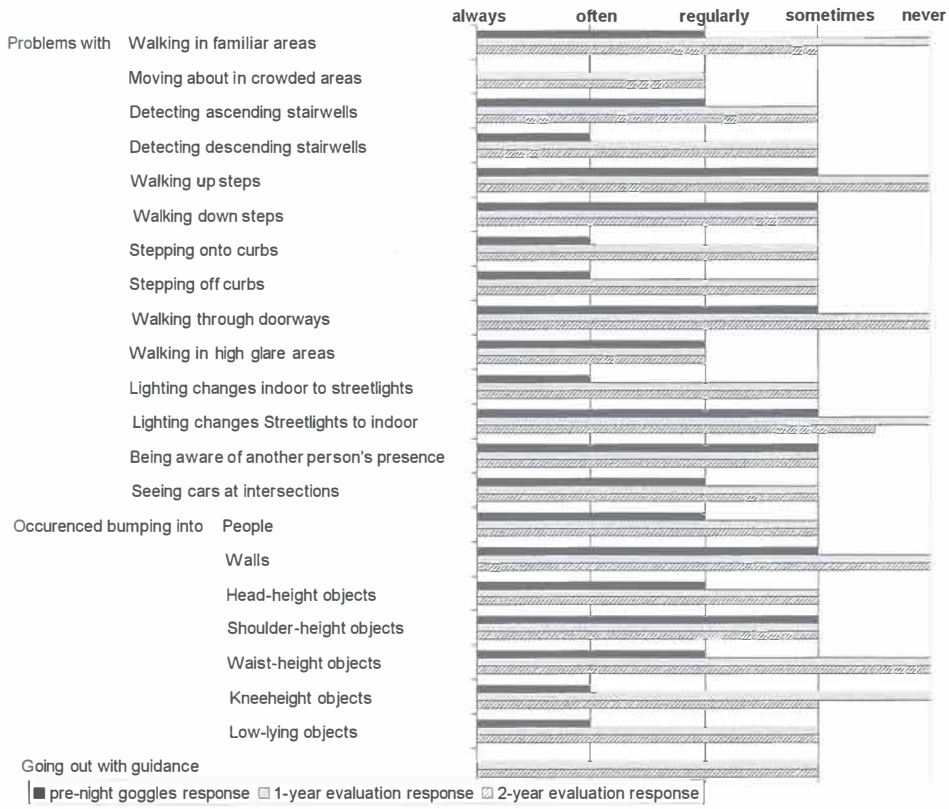


Figure 1. Median of answers to questionnaires regarding night-time mobility before using night-vision goggles (black bars), after 1 year (light grey bars) and 2 years (bars with diagonal stripes) of use for nine subjects who still used the device.

Use of night-vision goggles (period, frequency and purpose)

Night-vision goggles were used 'sometimes' in the spring and summer, 'regularly' in the autumn and 'often' in the winter (Figure 2). In the spring of the first year, the responses of only nine subjects are shown as two had not purchased the device at that time. In the winter period at 1-year follow-up, 4 (36%) of the 11 participants used the instrument daily, 2 participants used the instrument (18%) 2–4 times a week, 3 (27%) about once a week and 2 (18%) less than once a week. In the winter period at 2-year follow-up, 2 of the initial 11 subjects had stopped using the device altogether. Of the remaining nine subjects who responded on the questionnaires, two (22%) used the instrument daily, five (56%) 2–4 times a week and two (22%) less than once a week. Night-vision goggles were 'mostly' employed in 'familiar' environments and 'sometimes' in 'unfamiliar surroundings' by 71% (9 of 11) at year 1 and 78% (7 of 9) at year 2. Moreover, they were used for the following purposes: walks 'regularly' to 'always' by 9 of 11 (82%) at year 1, 6 of 9 (67%) at year 2, visit to

friends/family 'regularly' to 'always' by 5 of 11 (45%) at year 1, 4 of 9 (44%) at year 2, travel to work 'regularly' to 'always' by 5 of 11 (45%) at year 1, 5 of 9 (56%) at year 2, attending meetings 'regularly' to 'always' by 4 of 11 (36%) at year 1, 4 of 9 (44%) at year 2 and to a lesser extent for sports activities 'regularly' to 'often' by 3 of 11 (27%) at year 1, 2 of 9 (22%) at year 2. The reported reason that one of the subjects had stopped using the goggles was his move to a better-lit environment where he felt no need for the device. The other subject reported undergoing cataract surgery after which he saw more clearly, and he could then manage with just his torch.

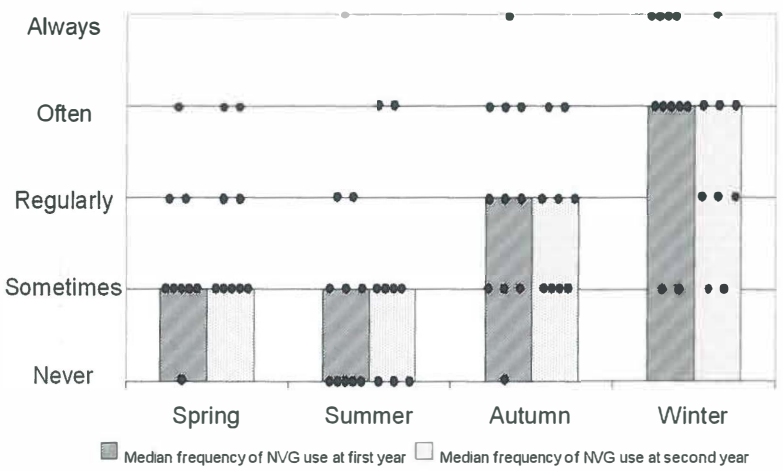


Figure 2. Individual responses (filled circles) and the median of the reported frequency of night-vision goggles use during each season at the 1- and 2-year follow-up.

Independence and experienced opportunities using night-vision goggles

Most of the individuals reported an increase in their independence at both the 1- and 2-year follow-up: 10 (91%) subjects at 1 year and the 9 (100%) who still used the instrument at 2 years. This variable was scored as 'much more independent' by 6 of 11 (55%) individuals at 1 year and by 6 of 9 (67%) at 2 years. It was scored as 'somewhat more independent' by four (36%) individuals at 1 year and three (33%) at 2 years (two had stopped). At both the 1- and 2-year evaluations, nine (82% at year 1, and 100% of responders at year 2) subjects reported that they went out alone in the dark more often since they started using NVG. At both 1 and 2 years, nine (82% at year 1, and 100% of responders at year 2) individuals also noted an increase in their opportunities for participation in activities: seven (64%) at year 1 and four (45%) at year 2 scored this as 'many more opportunities' and four (36%) at year 1 and five (56%) at year 2 as 'somewhat more opportunities'. For individual responses see Table 2.

Satisfaction with purchase/use of night-vision goggles

At both the 1- and 2-year evaluations, all (100%) subjects reported being satisfied with their 'purchase' of the instrument. Nine (82%) of the individuals at 1 year and five of nine responders (56%) at 2 years scored this item as 'extremely satisfied'. The remaining subjects two (18%) at 1 year and four (45%) at 2 years scored it as 'reasonably satisfied'. All subjects (100%) at 1 year and all who still used the instrument (100%) at 2 years indicated being satisfied with the 'use' of the goggles. Seven of 11 (64%) individuals at 1 year and 5 of 9 (56%) at 2 years scored this item as 'extremely satisfied', while 4 of 11 (36%) at 1 year and 4 of 9 (44%) at 2 years scored it as 'reasonably satisfied'. For individual responses see Table 2.

Limitations of night-vision goggles

At both the 1- and 2-year evaluations, median answers of the subjects with only small variation indicated that they 'sometimes' experienced limitations from the lack of depth perception at distance and nearby, a restricted field of view, screen disturbances and discomfort caused by an inappropriate fit of the NVG. Problems with light sources such as car headlights, varied widely between subjects from 'never' having problems to 'sometimes', 'regularly', 'often' or 'always' having problems.

Discussion

Night-blind subjects lack visual orientation in the dark and, if they go out, they regularly bump into objects on the streets. In practice, they do not go out alone when it is dark and are thus limited in their (independent) daily activities. Travelling to and from work or school in winter, attending an evening meeting or even taking a short walk for relaxation is challenging without the guidance of another person or an appropriate vision aid. In addition, an evening visit to a friend or family member in the neighbourhood cannot easily be performed spontaneously. In our previous paper (Hartong et al., 2004), we reported that a new version of the NVG clearly improved the mobility of night-blind subjects on dark outdoor residential test routes. In order to determine the effect of such a device on the daily life of night-blind subjects, we performed a 1- and 2-year evaluation of the practical use of the instrument using questionnaires.

Our results show that, after 2 years, two individuals had stopped using the instrument and two others used the goggles less than once a week. In contrast, the remaining seven subjects (64% of the initial group) used the instrument at least twice a week. This suggests that NVG can be integrated into the daily lives of night-blind subjects. The goggles were mainly used in the autumn and winter seasons,

which can be attributed to the presence of more hours of darkness per day (in the Netherlands, the sun does not set until 10:00 or 11:00 pm in the summer period, but in the winter period it begins to get dark at 4:00 to 4:30 pm).

The devices were 'regularly' to 'always' used to travel to and from work by five subjects at both 1 (45%) and 2 years (56%). Although the frequency differed between subjects and depended on the availability of a job, its location, and working hours (not all of the subjects had a regular job at the time of the study), the results, nonetheless, indicate that the use of NVG can decrease the limitations linked with a home–work commute. The same applies to attending evening meetings.

The nine subjects who still used the NVG at the 2-year follow-up indicated that they now often go out at night without guidance. In contrast, they 'always' went out in the dark with guidance before they had the device (see also Figure 1). The most important is that they feel more independent and that they have experienced an increase in their opportunities since using the goggles.

Although generally comparable, some small shifts in the individual evaluations can be seen between 1- and 2-year use of the NVG. Some showed a change from being 'extremely satisfied' with the purchase and use of the goggles to 'reasonably satisfied'. Yet, these same subjects did often report an increased shift in both independent activities and their sense of independence. At the 1-year evaluation, it was the first winter season in which the subjects used the instrument independently without study purpose. Perhaps the initial excitement has partly contributed to their extremely satisfied reports. A small decrease in frequency of use of the goggles is observed at year 2. An exact reason for this is not clear, but it might be that the subjects became more selective in the purposes for which they used the instrument. In half of the cases where the instrument was only used 'sometimes' for several purposes at the first year, it was not used for that purpose at the second year.

There are some limitations with regard to the use of NVG. Figure 1, for example, shows that, at both the 1- and 2-year evaluations, our subjects still regularly experienced problems with blinding light (e.g. car headlights, streetlights). This blinding might happen as a consequence of the strong amplification of the video camera signal causing local overexposure and blooming of the image presented on the two monitor displays. It will be an improvement if overexposure around a high luminance source can be avoided by future developments of the NVG. Our previous study (Hartong et al., 2004) showed that the mobility of night-blind subjects was normal in a shopping area with a high-illumination level and that

there was no need for a vision aid there. The instrument, therefore, should probably (temporarily) not be used in areas with many high-glare light sources. Our subjects also 'regularly' experienced problems while walking in crowded areas. However, this is a clear improvement to the situation without the use of NVG, when subjects 'always' had trouble in crowded areas. Other limitations, such as the lack of depth perception, image disturbances and inconvenience caused by a non-perfect fit of the goggles, were only 'sometimes' experienced as a problem.

This study did not compare NVG with previously known night-vision aids such as the wide-beam lantern or the cane. The three instruments are completely different kinds of vision aids, differing in costs, user options and, possibly in effect. A comparison between the three with the use of a long-term subjective evaluation would be interesting.

It should be noted that as our research group (11 subjects) was rather small, a selection bias might be present. Moreover, all of our subjects were committed to participating in the research and in buying NVG. They were, therefore, very well motivated to obtain a vision aid for their problems. This motivation may have led to a positive bias in results. It also indicates the great need of night-blind subjects for a vision aid. Although the majority of our subjects were extremely positive about the device, a few were only moderately positive; in addition two had now stopped using it. Their reasons were indicated as a movement to a better-lit environment and a cataract surgery. Both these subjects had reported a partly increased independence at the first year (see Table 2). However, they also indicated that they did not go out more often since using the goggles. It might, therefore, be that these subjects had no strong reasons to stick with their use of the device anyway. They did, however, declare being satisfied with their purchase of the device as they think they will need the goggles when their situation changes. Our previous study also noted three subjects who disliked using the device. This was not related to parameters such as visual acuity, visual field or age, but was probably more dependent on personal preferences. It should, however, be recalled that the visual fields of all of our subjects were all already severely constricted because of their disease. We therefore cannot make any statements on subjective evaluation by those who have larger visual fields.

This report on NVG should be brought to the attention of night-blind individuals. It shows that the goggles were evaluated positively and that they enhanced the quality of daily life of most of our night-blind participants. Night-blind subjects who feel a need for a vision aid should, therefore, be given the opportunity to test NVG.

In summary, the results indicate that NVG were still being used regularly by most of our night-blind subjects after 2 years. Moreover, the goggles had a positive effect on the subjects' experienced opportunities and sense of independence. The instrument was considered a useful tool in the daily lives of most of our subjects.

References

- Berson, E. L., Rosner, B., Sandberg, M. A., Hayes, K. C., Nicholson, B. W., Weigel-DiFrano, C. and Willett, W. (1993) Vitamin A supplementation for retinitis pigmentosa. Arch. Ophthalmol. 111, 1456–1459.
- Berson, E. L., Rosner, B., Sandberg, M. A., Weigel-DiFranco, C., Moser, A., Brockhurst, R. J., Eayes, K.C., Johnson, C. A., Anderson, E. J., Gaudio, A. R., Willet, W. C., Schaefer, E. J. (2004) Further evaluation of docosahexaenoic acid in patients with retinitis pigmentosa receiving Vitamin A treatment: subgroup analyses. Arch. Ophthalmol. 122, 1306–1314.
- Black, A., Lovie-Kitchin, J. E., Woods, R. L., Arnold, N., Byrne, J. and Murrish, J. (1997) Mobility performance in retinitis pigmentosa. Clin. Exp. Optom. 80, 1–12.
- Bowers, A. R., Luo, G., Rensing, N. M. and Peli, E. (2004) Evaluation of a prototype Minified Augmented-View device for patients with impaired night vision. Ophthalmic Physiol. Opt. 24, 296–312.
- Friedburg, C., Serey, L., Sharpe, L. T., Trauzettel-Klosinski, S. and Zrenner, E. (1999) Evaluation of the night vision spectacles on patients with impaired night vision. Graefes Arch. Clin. Exp. Ophthalmol. 237, 125–136.
- Geruschat, D. R., Turano, K. A. and Stahl, J. W. (1998) Traditional measures of mobility performance and retinitis pigmentosa. Optom. Vis. Sci. 75, 525–537.
- Hartong, D. T., Jorritsma, F. F., Neve, J. J., Melis-Dankers, B. J. and Kooijman, A. C. (2004) Improved mobility and independence of night-blind people using night-vision goggles. Invest. Ophthalmol. Vis. Sci. 45, 1725–1731.
- Kuyk, T., Elliott, J. L. and Fuhr, P. S. (1998) Visual correlates of mobility in real world settings in older adults with low vision. Optom. Vis. Sci. 75, 538–547.
- Morrissette, D. L., Marmor, M. F. and Goodrich, G. L. (1983) An evaluation of night vision mobility aids. Ophthalmology 90, 1226–1230.
- Rohrschneider, K., Spandau, U., Wechsler, S. and Blankenagel, A. (2000) Utilization of a new night vision enhancement device (DAVIS). Klin. Monatsbl. Augenheikd. 217, 88–93.
- Spandau, U. H., Wechseler, S. and Blankenagel, A. (2002) Testing night vision goggles in a dark outside environment. Optom. Vis. Sci. 79, 39–45.
- Turano, K. A., Geruschat, D. R., Stahl, J. W. and Massof, R. W. (1999) Perceived visual ability for independent mobility in persons with retinitis pigmentosa. Invest. Ophthalmol. Vis. Sci. 40, 865–877.

Chapter 7

Discussion:
Retinitis Pigmentosa and possible interventional strategies in perspective

Introduction

Alterations in our coded Deoxyribonucleic acid (DNA) may cause clinical problems from birth or may induce functional deficiencies in later years. So far, 141 genes and another 47 mapped loci have been associated with clinically detectable retinal disease (www.retnet.com). The majority of patients with inherited retinal diseases belong to the group of retinitis pigmentosa (RP): 55 of the identified genes have been associated with this disorder (including Usher and Bardet-Biedl syndromes). The identification of causative gene defects has contributed to a rapidly increasing knowledge of retinal function, but simultaneously raises many questions as to the complex pathophysiologic mechanisms.

Retinitis pigmentosa is extensively described in **chapter 1**. Briefly, the classical presentation consists of night-blindness usually starting around age 20, and progressive constriction of the visual fields. Although some patients retain some central visual acuity throughout life, the small remaining tunnel of vision is severely disabling and subjects are considered legally blind. For such a disabling disease, the main goal for the clinician is to provide an appropriate treatment. It is however disappointing to acknowledge that no curative treatment, at least for this whole group of disorders, has been achieved up to today. Yet, progress is being made for the treatment of selected mutations and for slowing down the disease process in a broader set of cases. The problems arising with respect to the search for accurate treatment and results obtained from animal and clinical studies will be addressed to in this discussion.

One of the main problems towards finding a treatment in RP considers the enormous diversity of causative gene defects, which is outlined in figure 4 and table 1 of **chapter 1**. Recently some progress is being made with respect to the identification of new genes that contribute to the development of RP, and include: Nuclear Receptor subfamily 2, groep E, member 3 (*NR2E3*)[1, 2], Retinol dehydrogenase 12 (*RDH12*)[3], Topoisomerase 1-binding argininge/sering-rich protein (*TOPORS*)[4] for autosomal dominant RP, Homolog of drosphila Eyes Shut) (*EYS*)[5-7], Isocitrate dehydrogenase 3, beta subunit (*IDH3B*) (**chapter 2**, this thesis), and Progressive rod-cone degeneration (*PRCD*)[8] for autosomal recessive RP. Prominin 1 (*PROM1*) has also been associated with recessive RP, but seems to be accompanied by macular degeneration[9]. An updated figure of contributing genes is shown in box 1 in the diagram of this chapter. The total of 55 causative genes identified so far are expected to represent only about 60-70% of the total genes responsible for the total group of RP. With exception of the Usher 2A (*USH2A*), rhodopsin (*RHO*), and Retinitis Pigmentosa GTPase regulator (*RPGR*) genes,

which each account for about 10% of total RP cases, the majority of genes each cause only a few percent of cases (see also box 1, diagram). Another exception may be a recently discovered novel RP gene, the *EYS*-gene, which corresponds to the RP25 locus[5, 6]. It involves the largest eye-specific gene known so far, and may potentially account for a relatively high percentage of RP-patients. When looking at the gene functions outlined in table 1, **chapter 1**, it is seen that the known genes are involved in different structural, functional and signalling retinal pathways, or may even consider defects in genes with non-retinal-specific general body functions. The latter of which our study in **chapter 2** is an example: loss-of-function mutations in the citric acid cycle enzyme 'isocitrate dehydrogenase' are associated with nonsyndromic recessive RP.
Interventional therapies may be focussed on different levels in the development of RP, ranging from the initial gene defect, the subsequent intermediate pathways that trigger photoreceptor rod cell death, factors that stimulate cell survival, or factors that cause the secondary loss of cone photoreceptors. The diagram added to this chapter may be used as a guide to read this chapter.

Gene therapy *(box 2, diagram)*
The ultimate cure would be a replacement of the defective gene before the secondary destructive effects take place. As is also explained in the future section of **chapter 1**, the best experience with respect to gene therapy is achieved with the autosomal recessive gene, Retinal Pigment Epithelium specifice protein, 65KD (*RPE65*), that causes a retinal degeneration that is related to retinitis pigmentosa but causes a more severe vision loss from early life, and is termed Lebers congenital amaurosis [10]. Recently, phase I clinical trials using subretinal adeno-associated virus (AAV) injections, have been performed and reported no surgical complications and no immunological problems[11, 12]. The studies were too preliminary to draw any definite conclusions about the changes in visual function, but one person in both studies showed improved navigation. Further studies with more patients are needed to determine the actual effect on visual function and patient safety. The possible by-effect of non-gene related factors such as neurotrophic factors needs to be established, as do the optimal dose, and appropriate interventional time frame.

Although the therapy seems promising, even when it appears to become a well-tolerated effective intervention in the case of *RPE65*, the method cannot simply be transferred to all other gene defects. First of all, there needs to be a loss-of-function mutation as is often the case in recessive mutations, for which the introduction of a healthy gene could solve the original problem. In any other case, such as for most dominant mutations where an altered encoded protein influences downstream

pathways, or produces toxic by-products, the insertion of a healthy gene will not or only partially stop the mechanism that ultimately leads to the photoreceptor cell death. This problem may be solved by the simultaneous introduction of a gene-silencing product as ribozymes [13, 14] or ribonucleic acid interference (RNAi)[15, 16] that prevents the production of the misformed protein and thus prevents the altered molecule from practising its detrimental secondary effects. Mutation specific gene silencing will only block the production of the mutated gene product and will allow normal translation of the wild type allele. The 50% production of normal protein may be sufficient for (sub) normal function and may therefore prevent the need of simultaneous gene replacement[17]. A novel strategy for splice site mutations involves the introduction of adapted U1 small nuclear RNA (U1 snRNA) which should correct misspliced mutations[18]. However, as in the case of the autosomal dominant Rhodopsin gene *(RHO)*, where over a hundred different RP-causing mutations have been described (RetNet Database), a mutation dependent strategy is not very efficient and a mutation independent strategy may be preferred. With this strategy both the mutated and the wild type gene will be blocked. In this case, a replacement gene should be introduced and designed in a way so that it is resistant to the silencing product. A potentially broader applicable method is antisense oligonucleotide exon skipping of one or more exons that may contain numerous mutations. The method aims to result in an abberant protein in stead of a truncated protein that is otherwise formed through the formation of a premature stop codon. In the case of Duchenne's muscular dystrophy, the targeted skipping of exon 51 led to dystrophin synthesis in four patients[19]. The rescuing effect will however be dependent on the protein and the specific function of the skipped nucleotides, and may not be applicable to many of the (smaller) genes implicated in retinitis pigmentosa.

In general, the eye seems a suitable organ for targeted gene therapy since viruses can be injected locally under direct visualisation and they efficiently transfer to the target tissue. Furthermore, the blood-retina barrier prevents widespread dissemination of the virus injected. The effectiveness can be determined with visual function tests[20]. However, extensive animal studies will be needed to address all issues that are faced by the specific gene therapy. In the end, if a method seems potentially successful and safe in animal studies, the method should be tested in humans. Clinical trials with sufficient volunteers will be needed to determine the safety and effectiveness of the gene transfer, which in the case of rare mutations may not be easy. Another difficulty will be the determination of the appropriate interventional time window. In the case of Leber congenital amaurosis, the photoreceptor layer is relatively well preserved for a considerable period after the visual function has almost been lost. In contrary, in the case of typical RP,

many or most patients will remain some visual function throughout their whole life. The timeframe where sufficient healthy photoreceptors are available for an appropriate intervention may be in a period with still relatively well preserved visual function, and may make the decision to participate in a human clinical trial difficult.

It should be clear that all the time, effort and costs may not be worthwhile for the majority of RP genes that only cause a few cases. The exception with respect to patient numbers may be the recessive *USH2A* and *RPGR* genes, as well as the dominant *RHO* gene, and possibly the newly discovered EYS gene, which each affect about 10% of the total RP population. With rhodopsin-linked mice models some functional results have been obtained using the suppression-replacement technique[15, 21] and may thus form a potential option. If gene therapy appears to become a safe and efficient method, the application may be transferred to a more general mutation-independent approach, where genes could be introduced that for example promote cell survival and could thus be used for an extended number of RP patients.

Another interesting direction with respect to intervention on the genetic level involves the pharmacological intervention in premature termination codon mutations. Aminoglycosides seem to induce ribozymes to read-through a premature stop codon, so that a full-length protein will be formed. Experiments have indicated that aminoglycosides may provide in an (temporary) increased level of full-length proteins with photoreceptor rescue effect on some, but not on other animal or human cell line models [22, 23]. Caution is warranted with respect to the toxic systemic side effects of these antibiotics. A non-aminoglycoside drug called PTC-124 (Ataluren), was developed to induce ribosomes to read through premature stop codons, but not normal stop codons[24]. The drug has been tested in cystic fibrosis patients and seemed to improve the chloride transport function[25]. Whether this drug could be used in the treatment of retinitis pigmentosa resulting from premature termination codon mutations should be explored.

Retinal transplants and electrical stimulation *(box 2, diagram)*

Retinal transplants are also not dependent on the specific gene mutation and are potentially able to restore visual function. Photoreceptor precursors seem to be capable of developing into mature photoreceptor cells with integration in the mouse host retina and possible improvement of visual function[26, 27]. Further experiments are focussed on the generation of photoreceptors from stem cells. Another approach is to transplant retinal progenitor cells together with the retinal pigment epithelium (RPE). A phase II clinical trial where retinal/RPE tissue from aborted fetuses were transplanted in eyes of patients with RP or dry age-

related macular degeneration (AMD) was completed: three out of ten RP patients, and four out of four AMD patients showed modest improved visual acuity in patients with 20/200 or less initial visual acuity[28]. However, a lens extraction with intraocular lens implant and surgical capsulotomy was also performed in all transplant eyes, impeding the interpretation of results. Also, a potential bias may have occurred since the subjects were not blinded to their treatment. Results should thus be interpreted with caution. Subsequent studies with larger cohorts will be needed to determine its effect and exclude the possibility of activation of neurotrophic factors. It may be preferred to locate the transplants more under the foveal area in order to aim for an improvement of visual acuity of more than 20/200. The technique may not be the first choice in patients with a relative well preserved visual acuity. Yet, when these techniques would ever achieve its functional application in humans, it could become an important tool, since it may not only help RP-patients with all their different mutations, but also other retinal degenerative diseases like age-related macular degeneration or Stargardt's disease.

Electrical stimulation studies are currently being pursued. A lot of obstacles still have to be overcome, see the review by Bertschinger[29]. These interventions will however, when applicable, probably only be an option for end-stage disease.

Cell death pathways in retinitis pigmentosa *(box 3 and 4, diagram)*
In order to look for interventions that may help a more general group of RP patients, it may be better to define common elements that influence the course of pathways occurring in the total group of RP mutations.

The diagram in this chapter outlines (part of) the mechanisms known to contribute to photoreceptor cell death with possible interventional strategies as presented in the literature. Animal models with distinct RP-causing mechanisms were used to obtain much of these data. Hereditary animal models that often have been used include 1) the retinal degeneration *(rd1)* mouse [30] which has a null mutation in the cyclic guanosine monophosphate – phosphodiesterase (*cGMP-PDE*) gene [31, 32]. These mice lack the beta subunit of *PDE* which normally inactivates cGMP. The resulting increased conductance through cGMP gated channels causes a massive influx of sodium (Na+) and calcium (Ca2+) ions which causes a metabolic overload and possibly direct toxicity from increased Ca2+ levels. 2) Retinal degeneration slow *(rds)* mice [33] lack the *RDS/peripherin* gene, which encodes a structural membrane bound glycoprotein expressed in both rods and cones. Heterozygous mice partly fail to form photoreceptor outer segments and their nuclei undergo apoptosis but in a later stage than the rd mice. 3) Royal college of surgeons (RCS) rat [34, 35] have a defect in the human homolog Mer tyrosine kinase protooncogene

(*MERTK*) and causes impaired phagocytosis of the shed photoreceptor outer segments. A non-hereditary inducible model that is often used is the light-induced retinal degeneration model [36]. Two types of light induced retinal degeneration exist: one occurring in bright light where apoptosis occurs independent of transducin, and is accompanied by activator protein 1 (AP-1), a transcription factor. Low light induced retinal degeneration is transducin dependent and is likely the result of excessive phototransduction signalling. More recently, many transgenic mouse (and other animal) models that mimic the human mutations have been created[37].
A longer accepted consensus between study groups that work on different animal models is the fact that apoptosis (programmed cell death) is a shared final common pathway [38, 39]. Yet, more recent studies point to the existence of other parallel final pathways including autophagy and necrosis[40, 41]. Apoptotic mechanisms can be defined as caspase-dependent (mostly caspase-3) and caspase-independent, including those mediated by Apoptosis Induction Factor (AIF), Endonuclease G (endo G), Poly- (ADP-ribose) polymerase 1 (PARP-1), Ubiquitin-proteasome system, and lysosomal proteases[42]. It is also suggested that it may be the autophagy pathway that initiates apoptosis and that therapeutic options should be aimed at blocking autophagy[41].

Interference in cell death pathways: neuroprotection *(box 4, diagram)*
Neuroprotective factors, such as Basic Fibroblast Growth Factor (bFGF) and Ciliary neurotrophic factor (CNTF) interfere with apoptotic pathways and addition of these factors seem to promote cell survival. About a dozen of these factors have been injected intravitreally, inserted trough encapsulated technology, or were delivered through a viral transgene approach in the eyes of different animal models with retinal degeneration and have been nicely reviewed by Wenzel et al[42]. Results have often pointed to significant photoreceptor layer preservation, but many neurotrophic factors appear not to be effective in all models of retinal degeneration[42]. A phase I human clinical trial with encapsulated cell transfer of CNTF has successfully been completed with no serious adverse effects[43]. Also with this factor, the therapy might not apply to all gene defects, as was indicated with the Retinitis Pigmentosa GTPase Regulator (*RPGR*) dog where central and mid-peripheral photoreceptor rescue could not be achieved and instead extensive peripheral remodelling occurred[44]. Particularly interesting and in contradiction to what was expected, one study found that the heterozygous knockout of the Brain-derived neurotrophic factor (BDNF) gene in mice protected photoreceptors from light-induced degeneration. This effect is likely induced by an adaptive response including the observed upregulation of Glial cell derived neurotrophic factor (GDNF)[45]. So, it seems that an autoregulation, probably within certain limits, will provide a protective element, at least in the case of an otherwise healthy retina

that is only photochemically damaged. It will be difficult to predict the effect of neurotrophic factors in diseased retina, since the initial gene defect may already have induced an alternate sequel of events, or remodelling effect that may react differently in the multiple gene defects. Early intervention may possibly circumvent this problem. A phase II human clinical trial is currently being performed in subjects with early and late stage disease using intraocular encapsulated cell technology (ECT) implants (www.clinicaltrials.gov). Results of this clinical trial are awaited. Recently, another potential neuroprotective agent 'topical alpha2-agonist' (brimonidine) was evaluated in RP patients. The rationale of this study was that the drug seems to protect ganglion cells and preserves electroretinogram (ERG) b-wave amplitudes in experimental ischemia, and ameliorates light-induced ischemia, possibly by up regulating neuronal survival factors such as BDNF and bFGF. In this pilot study a trend for reduced visual field loss was observed for the treated eye, compared to the fellow untreated eye, but without statistical significance. The study population (n=26) was rather small and the follow-up period (2-3 years) was rather short. Also, a possible effect on the contra-lateral eye complicates the interpretation of the study[46]. Further studies with larger cohorts and longer follow-up time are needed.

Another suggested interference in the cell death pathway considers the application of minocycline which seems to inhibit caspase-3 activation and reduced photopic injury in light-induced retinal degeneration in rats[47].

Triggers of cell death pathways

Late triggers (box 5, diagram)

Studies have pointed to evidence of upregulation of oxidative stress genes[48, 49], an increase in glycosylation[40], an increase in complement factors[40], microglial activation[50], and calpains[51] all which seem to precede the final cell death pathway signs. In bright light induced retinal degeneration, the transcription factor AP-1 is upregulated[52]. Suppression of this factor and subsequent suppression of photoreceptor degeneration in mice exposed to diffuse bright light is achieved by subcutaneous dexamethasone injections[53]. Microglia are the primary cells in the active immune defence of the central nervous system, and they upregulate proinflammatory and neurotoxic factors, such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α. Nalaxone inhibits microglial activation and reduces photoreceptor degeneration in light-exposed rats[54].

A potential therapeutic endogenous antioxidant with effects on oxidative stress genes, considers the precursor of neuroprotectin D1 (NPD1) [56, 57]

which is derived from docosahexaenoic acid (DHA) in the retinal pigment epithelium. NPD1 seems to protect against oxidative stress induced apoptosis, through counteracting with TNFα, upregulation of anti-apoptotic genes (Bcl-2 (B-Cell/CLL lymphoma-2), Bcl-xL (Basal cell lymphoma extra-large)), downregulation of proapoptotic (Bax (Bcl-2 associated X protein), Bad (Bcl2 associated death promotor)) gene expression, inhibition of caspase-3 activation, and inhibition of stimulated cyclooxygenase-2 (COX2) expression.

A distinct apoptotic triggering mechanism occurs with autosomal dominant rhodopsin mutations encoding misfolded rhodopsin that aggregates in the endoplasmic reticulum. There is evidence of the initiation of the unfolded protein response (UPR) with impairment of the ubiquitin proteasome system (UPS)[58]. The response may be halted by addition of pharmacological chaperones that assist correct folding of the protein[59].

Early triggers *(box 6, diagram)*

Among the suggested early triggers of photoreceptor cell death, and thus potential efficient targets to interfere with, are calcium overload, metabolic stress, the formation of reactive oxygen species, and the formation of nitric oxide. At least in several models (light-induced degeneration, some RHO mutations, cGMP-PDE mutations), a massive calcium influx results from prolonged opening of cGMP gated channels[60]. The application of the calcium channel blocker diltiazem however did not rescue photoreceptor cell death in different retinal degeneration animal models[61-63]. Other studies suggest that nilvadipine rather than diltiazem may slow retinal degeneration in rd mice, rds mice and RCS rats [64-66]. It is however not clear whether this effect is obtained by molecular modulation of calcium levels or whether this is caused by the observed changes in expression levels of genes that influence survival of neuronal cells[64, 66].

Our discovery of mutations in the Kreb's cycle enzyme isocitrate dehydrogenase in RP patients, described in **chapter 2**, indicates that in some way perturbations in this 'oxygen consuming energy generating cycle' will either directly (by producing an insufficient amount of Adenosine triphosphate (ATP) for the highly active photoreceptors) or indirectly (by producing toxic by-products like oxygen radicals induced by the defective machinery of photoreceptor cell metabolism) affect the viability of photoreceptors. Further studies should address this issue. For example, the generation of a knockout mouse may give additional information. An important question to explore may be why rod photoreceptors are affected primarily. It seems that cones have a higher mitochondrial concentration and higher ATP-production in comparison to rods[67]. So, what will then explain the increased vulnerability in

rods? What are the differences in energy metabolism between rods and cones? Since metabolic stress also seems to be implicated in other models of RP, exploring these questions may give us a better insight for different RP mutations. One possibility is suggested by the work of Johnson et al., where it is described that mitochondria and calcium channels interact to regulate ATP production and calcium homeostasis at their synaptic terminals. It seems that the cellular components are distributed differently between rod and cone inner segments. For example rods have one large ovoid mitochondrion near its active synaptic terminal, while cones have on average 5 smaller mitochondria clustered away from the synaptic terminal. It is suggested that such structural differences may make the rod more susceptible to calcium overload[68]. Another subject of interest is the likely reduced production of alpha-ketoglutarate, since this is a product of isocitrate dehydrogenase activity. Alpha-ketoglutarate is involved in glutamate metabolism, the neurotransmitter in photoreceptors, and may therefore also be involved in the development of RP. Furthermore, may other Krebs cycle enzymes be involved in RP as well and these should be explored further.

The involvement of reactive oxygen species among the early triggers of apoptosis in neuro-degeneration was suggested by Carmody et al[69, 70]. Reactive oxygen species (ROS) are the product of normal oxygen metabolism, but in the presence of environmental stress, the ROS may accumulate in a condition known as oxidative stress, and the increased ROS may damage the cell and its DNA and proteins. Oxidative DNA damage in rods also seemed to be present in the rd1 mouse model of retinitis pigmentosa[49], and the same group demonstrated a slowing of the degeneration using a combination of antioxidants (lutein, zeaxanthin, alpha lipoic acid (ALA), reduced L-glutathione (GSH)), while individual antioxidants showed no significant effect[49]. A common element that seems to fit several animal models, and which may (in part) explain the occurrence of oxidative stress, is the increase in choroidal oxygen tension. Hyperoxia may in theory result from 1) shortening of the photoreceptor outer segments, with resulting decreased oxygen consumption and simultaneously closer proximity of the photoreceptor cell bodies to the oxygen-rich choroid. 2) the reduced oxygen need after massive photoreceptor cell death for which the retinal vascular autoregulation component (constriction of retinal arteries) is insufficient.

Some groups speculate that photoreceptor cell death results from hyperoxia in RP. The hyperoxic state of the outer nuclear layer was confirmed by Yu et al in the RCS rat and in the P23H rat[71]. However, a group from Spain hypothesized the opposite[72]. This group suggested that since photoreceptors use large amounts of energy, perhaps a better retinal metabolic efficiency might be achieved when

hyperbaric oxygen therapy is supplied to the patient. Results from their clinical trial where patients with hyperbaric oxygen therapy were compared to controls suggest a better preserved visual acuity, visual field en ERG-response. A major drawback of this study is that the tests could not be blinded, and that the objective ERG-response may be a functional response to the hyperbaric oxygen. Results should thus be interpreted with caution.

A clinical trial of antioxidant therapy in 34 adult patients with RP was performed by dietary supplementation of the macular carotenoid pigment lutein. The trial was a double-masked randomized placebo-controlled trial with a cross-over design (2 x 24 weeks). The results suggest an improvement of visual field and possibly a reduced decline in visual acuity and contrast sensitivity with lutein supplementation. Further studies should confirm these results since the study group was rather small and possible interference of results may have occurred by a delayed effect of lutein, lack of a washout period, the concomitant use of multivitamin supplementation, and a short study period[73].

A clinical trial where the effects of anti-oxidants for photoreceptor protection are evaluated is currently being performed among patients with geographic atrophy resulting from macular photoreceptor degeneration[74]. Age-related macular degeneration (AMD) is believed to result from a combination of genetic, and environmental factors. The role of antioxidant damage in AMD is suggested by a beneficial effect on disease progression with the use of supplemental[75], or higher dietary antioxidants[76]. Oxidative damage has also been detected in the retinas of postmortem patients with widespread geographic atrophy[77]. A common cell death mechanism may thus be involved in both geographic AMD and RP. However, differences in etiology will complicate the translation of results to RP. In stead of nutritional intervention, the trial uses local antioxidant eyedrops, of which it may be hard to determine whether it will reach sufficient levels at the posterior part of the eye.

Less explored, but potential interesting drugs are 1) the hormone estrogen, which may act through calcium homeostasis, enhanced citric acid cycle driven glycolysis, protection of mitochondrial function and free radical damage[78]. One study showed that in the rhodopsin Ser344Ter model of retinitis pigmentosa, an estradiol analog protected against retinal degeneration[79]. However, a great restraint is warranted as it seems that estrogen supplementation in diseased neurons may be detrimental because of the presence of dysregulated calcium homeostasis[80]; 2) a novel drug tested on the rp10 mouse with resultant preservation of photoreceptors considers Tauroursodeoxycholic acid (TUDCA), a drug

which has longer been used in Chinese medicine and is possibly involved in mitochondrial stabilization, anti-oxidation, and blockade of caspase-3 activation [81]. Whether or not autoimmunity plays a role in RP is not well understood. Cyclosporin eye drops (0.5%) are currently being evaluated in a clinical trial in order to look for the potential involvement of autoimmunity in RP[55]. Apart from being an immuno-modulating drug, cyclosporin A blocks the formation of the mitochondrial permeability transition pore, thereby preventing the release of the apoptosis inducing factor cytochrome-c, and may thus be protective in a non-immune mediated way.

Interference in visual cycle *(box 7 and 8, diagram)*

While most of the previous data were obtained through animal studies, Berson and his group have used the clinical approach: an initial study on the natural course of RP suggested a protective effect from supplemental vitamin A and E[82]. A subsequent randomized clinical trial indicated a protective effect in adult RP patients of high dose oral vitamin A palmitate, but a deleterious effect from vitamin E[83]. The protective effect was revealed by a modest delay in retinal degeneration recorded by cone-electroretinogram[83] and in a selected group also on the visual field[84]. For more details, the reader is referred to **chapter 1**. Vitamin A supplementation (15 000 IU of oral vitamin A palmitate) is the only recommended supplemental therapy in the United States up to today. Care should however be taken to avoid a total intake greater than 25 000 IU, since these doses can be potentially toxic. Patients should thus be monitored for liver function, and elderly patients must be monitored also for bone health. It should also be noted that the protective effect was on average across the whole group of tested RP subjects. From the study it could not be predicted which mutations may or may not benefit from vitamin A therapy, since the sample sizes of different mutations were too small. While many subjects are thus expected to benefit from vitamin A therapy, some may not, or may even deteriorate from the therapy. Individual predictions for a therapeutic effect over the long term can thus not be made, except that the rate of decline may be followed and the drug may be stopped if the decline appears to exceed the expected course. Yet, the tools to detect these deviations will not be readily available in many clinics. It has been suggested that dietary supplementation with vitamin A may be deleterious for RP caused by the ATP-binding cassette, subfamily A, member 4 (*ABCA4*) mutation[85, 86]. ABCA4 is involved in the transport of vitamin A in the photoreceptor rod and cone outer segments [87, 88]. Blockade of vitamin A in mice resulted in decreased accumulation of toxic lipofuscin fluorophores in the retinal pigment epithelium, and likewise the supplementation of vitamin A in the diet of ABCA4 knockout mice resulted in the accumulation of toxic lipofuscin fluorophores in the retinal pigment

epithelium, but photoreceptor degeneration was similar between supplemented and non-supplemented mice over a period of 11 months[86]. Despite no observed difference in photoreceptor degeneration, caution should be taken because of the increased accumulation of toxic by products after vitamin A supplementation in ABCA4 mutations. In the Netherlands, patients are advised to exclude the ABCA4 mutation before starting supplementation with vitamin A therapy.

Furthermore, in the case of light-induced retinal degeneration, a blockade or slowing down of the visual cycle seems to protect the retina: the retina is protected against light-induced damage when the visual cycle is blocked in the case of RPE65 knockout mice (due to a lack of visual pigment regeneration)[89], or when the visual cycle is slowed as occurs with variations in the RPE65 gene[90]. Similarly, the pharmacological blockade of the visual pigment by halothane (which competes with 11-cis retinal for binding to opsin molecule) protects against light induced damage[91], as does the addition of the pharmacological drug isotretinoin (13-cis retinoid acid)[92]. However, since light-induced degeneration is specifically dependent on the amount of visual pigment, it is not clear what this will mean for the natural course of inherited degenerations, but it may be wise to test the effect of vitamin A therapy in different models with inherited gene defects in the visual cycle.

From the patients in the vitamin A study, it was also observed that those with high red blood cell docosahexaenoic (RBC DHA) levels had a significantly slower decline in ERG amplitudes. DHA is found to be highly concentrated in photoreceptors and retinal synapses, and peripheral blood and photoreceptor DHA levels are decreased in RP[57]. The retinal pigment epithelium recycles DHA back into the inner segments during outer segment renewal[57], and retinal function seems to depend on adequate levels of DHA in retinal lipids[93]. A randomized trial that tested the effect of DHA capsule supplementation, in addition to vitamin A therapy, did however not record a significant effect on the course of the disease[94, 95], except for those that started on vitamin A therapy. The beneficial effect of DHA capsules was lost two years after the start of vitamin A therapy. However, a subgroup analysis did point to a beneficial effect in those using a diet rich in omega-3 fatty acids (1-2 servings of omega-3 fat fish per week) and who had increased levels of RBC DHA[96].

Cone photoreceptor protection (box 9, diagram)

There is substantial evidence that, at least in some forms of retinitis pigmentosa, rod photoreceptors die initially and cones die secondarily after the loss of rods. This implies that the cones are either dependent on healthy rods, or that the cones eventually die from toxic factors produced by massive numbers of dying rods. The

first is supported by the identification of a rod derived cone viability factor[97]. The toxic effect was proposed by Ripps who speculated that the death of cones was a 'bystander' effect after receiving apoptotic triggers through intercellular gap-junctions [98]. Gupta et al. suggested that microglia activated by dying rods migrate to the outer nuclear layer to clear cellular debris and secrete toxic substances that kill nearby cells including cones.[50] Shen et al. described a toxic effect of oxidative stress mediated by dying rods contributed to cone cell death[99]. It may be that the cone photoreceptors die secondarily from the toxic reactive oxygen species resulting from hyperoxia[99]. Protection of this oxidative damage should thus prevent the loss of cone photoreceptors. Slowed photoreceptor degeneration after treatment with antioxidants was observed in different mice models, suggesting that the strategy might be broadly applicable[49, 100]. The same group has also found evidence for peroxynitrite-induced nitrosative damage. Peroxinitrite is generated from nitric oxide (NO) and with intraperitoneal injections of nitric oxidase inhibitors (NOS) an increased cone survival was observed in the rd1 mouse model of RP[101].

A hypothetical explanation for the benefit of vitamin A and DHA proposed by Berson is that in daylight conditions the visually inactive rods may serve as source of retinoids for the visually active cones[102]; With retinal light exposure in daylight, the rods will photo-excite as well. After excitation of the 11-cis-retinal retinoid in the rhodopsin molecule, a conformational change occurs and all-trans-retinol (a form of vitamin A) is formed. The known visual cycle involves the regeneration of rod photoreceptor retinoid (configuration of all-trans-retinol to 11-cis-retinol to 11-cis-retinal) via the retinal pigment epithelium, see also figure A (diagram). Mata et al. demonstrated an alternate cycle in cone-dominant ground squirrel and chicken retinas. In these cone dominant retinas, all-trans-retinol (vitamin A) is converted to 11-cis-retinol within the nearby intra-retinal Mueller cells. After transport of 11-cis-retinol to the cones, the cones further convert 11-cis-retinol to 11-cis-retinal, the product needed for visual excitation[103], see also figure B (diagram). Berson hypothesized that in daylight conditions the rods may supply all-trans-retinol (vitamin A) to the Mueller cells and thus indirectly serve as the retinoid source for the highly active cones in daylight conditions. If this is true, the cones' source of vitamin A would be depleted in retinitis pigmentosa after the rods have degenerated. The cones may die secondarily from this lack of vitamin A, and therefore the supplementation of vitamin A may delay the secondary death of cones. And as for the function of DHA, the retinoids are transported by the interphotoreceptor binding protein (IRBP) and its release is dependent on DHA[104]. So, a lack of DHA and vitamin A may worsen the course of secondary cone photoreceptor degeneration. Future experiments that focus on this interesting hypothesis should be encouraged.

Severity factors

The genetic predisposition to obtaining disease is evident from the Mendelian inheritance patterns in affected families. However, as the paper on telomere length (**chapter 3**) also describes, other factors besides the primary pathogenic mutation are expected to contribute to disease severity. If we are able to define these factors, this may help better understand cell death pathways, and most importantly may provide targets for therapeutic intervention. From age-related diseases we learn that it likely is the genetic background against which a person is more or less prone to develop disease with the final common pathway of cell death. With our study on telomere length we hypothesized that telomere length might be a contributing factor, since telomeres have previously been associated with apoptosis and disease severity. From our measurements of telomere length in leucocytes, we found no association between telomere length and severity of disease. Whether perhaps variations in the telomere genes (Telomerase reverse transcriptase (*TERT*)/ Telomerase RNA component (*TERC*)) or the telomere stabilizing proteins (Telomeric repeat binding factor 1 (TRF1)/(Telomeric repeat binding factor 2 (TRF2)), or changes in their expression profiles may contribute to disease severity should be explored further. Other suggestions would be Single Nucleotide Polymorphysms (SNPs) associated with a higher change of developing other retinal diseases such as age-related macular degeneration, or SNPs in pro- and anti-apoptotic genes.

Future directions

In the near future, it is possible that some patients with RP may be treated with specific gene therapy, and perhaps even more patients may be treated with drugs or other agents to preserve cone function. Both vitamin A and antioxidant therapy might contribute to the cone cell survival (Box 9 in the diagram). Potentially these regimens may together amplify their effect on cone survival. It should however first be further explored in animal models which antioxidants, and which combinations will exert the best influence on cone survival. Other regimens that may work additively are insertion of genes encoding neural survival factors (e.g., rod derived cone viability factor), and an omega-3-fat rich fish diet that should exert its protective effect trough DHA (involved in the release of vitamin A, and involved in the regulation of oxidative stress genes, and the blockade of caspase 3). Since microglial activation is also suggested to play a role in the secondary death of cones, the effect of Nalaxone should be explored further. Interference in calcium homeostasis may in theory still play an important role as calcium overload has been implicated in several RP models, and intracellular calcium levels seem to regulate

several photoreceptor pathways, as is also indicated in figure C. The calcium channel inhibitor Nilvadipine has been associated with photoreceptor survival and should therefore be explored further. The drugs TUDCA and estrogen (derivatives) may be interesting partly because of their potential function as anti-oxidants and in the case of estrogen because of its influence on calcium homeostasis. However their effects on mitochondrial metabolism and it's implications for RP are as yet unclear and should first be explored further. Ongoing research to detect factors that influence the severity of disease should be encouraged, as well as the search for new genes and their functions, in order to better predict who may or may not benefit from certain treatment strategies. If clinical trials are to be performed, they should be well organized, preferably multi-centered, masked, placebo controlled, and with a sufficiently long follow-up period to detect modest benefits. A definite cure is however not likely to be available within a short time period. Even with agents to preserve cone function, the majority of patients are likely to experience visual function problems throughout their lives. In the mean time, we must not forget the sources already available to support the visually disabled patient, see section below.

Vision aids to improve mobility and independence in RP patients

Ophthalmologists should be aware of the possibilities and developments regarding visual rehabilitation that are often provided at local, specialized, low vision centers or visual rehabilitation institutes. Apart from providing psychological support and educational and occupational related advice, a lot can be achieved in terms of improving visually related performance. In the Netherlands three main rehabilitation institutes exist; Royal Visio National Foundation for the Visually Impaired and Blind in the Netherlands, Sensis and Bartimeus, which together accommodate over 40 regional institutes. These institutes aim to serve each patient's individual demand for health service. In the experience of these institutes, the most frequently encountered request from RP patients is to improve their independence in dark or unknown environments (Vrijling et al, in process).

Chapter 5 has addressed the application of a light-amplification night vision device in RP patients. The first tests showed a direct improvement of night-time mobility, which even improved further after a few sessions of orientation-and-mobility training. Subjective evaluation showed that the majority of study patients experienced an improved sense of independence. Furthermore, as described in **Chapter 6**, from the subjective evaluation after two years of those that had actually bought the night-vision device, it appeared that seven (out of 11) still used the goggles at least twice a week. Results show the device could thus be implemented into the daily lives of these patients and that the use of the goggles improved their

sense of independence. Another study tested a different night-vision viewer and compared the results with a Wide Angle Mobility Lamp (WAML). Both instruments improved independent nighttime mobility with an overall better performance with the WAML, but some patients preferred the night-vision viewer[105]. An earlier study did also compare these mobility aids, however a handheld portable device with limited resolution was used and therefore not comparable to the contemporary devices[106].

The studies induced the national rehabilitation institutes to start an implementation project for night-vision aids, with the aim to accurately advise their clients with respect to their requests for independent night-time mobility (Vrijling et al, in process). Not only the night vision goggles were included, but also a broad angle flash light and head-LED lights. The studies described in **chapter 5 and 6** indicated that visual function measurements did not correlate to mobility-improvement or subjective satisfaction. A prediction about which night time mobility aid is superior can thus not be made from the visual function parameters. Therefore all subjects with an impaired dark-adaptation of at least 2 log units, irrespective of their visual function or diagnosis, were offered the three types of night time mobility aid. The participants became familiar with the mobility aids at the first visit, where a low vision specialist demonstrated the devices and gave the patients the opportunity to use the instruments. All subjects picked a first and second choice device, and with these two instruments the subjects could individually practice in their own environment. In addition, weekly mobility training was provided by mobility-trainers from the local rehabilitation center. At the last visit, all aspects including finances are individually discussed.

With this setup, all participants (n = 30) felt that they could make an informed decision as to their preferred night vision aid. Twelve participants chose the night vision goggles as their preferred mobility aid, seven chose the mobility lamp, four preferred the head-LEDs, and seven decided they were better off without a night-vision aid. Importantly, all felt their help requests were considered and answered. The night vision goggles have recently been included in the list of night time mobility aids in all Dutch rehabilitation institutes. The protocol 'guidance for night time mobility aids' as summarized above has now been implemented in these centers.

Figure C, diagram: insight in the phototransduction cascade

As is outlined in **chapter 1 and 4**, the phototransduction cascade is an important pathway involved in retinal function, and mutations in six genes involved in the phototransduction cascade are known to cause retinitis pigmentosa (RHO, cGMP specific phosphodiesterase 6A alpha subunit (PDE6A), cGMP specific phosphodiesterase 6A alpha subunit (PDE6B), (cylic nucleotide gated channel alpha 1 (CNGA1), cyclic nucleotide gated channel beta 1 (CNGB1), S-antigen(SAG)/ arrestin. In a normal phototransduction cascade, the transmembrane-bound rhodopsin (RHO) molecule is activated by a photon whose energy induces a conformational change of the visual pigment (11-cis-retinal) that is covalently linked to opsin. This activation allows rhodopsin to "turn on" transducin by releasing transducin's alpha subunit. Activated transducin in turn activates a cGMP-phosphodiesterase (PDE6). Activated PDE6 converts cyclic guanosine monophosphate (cGMP) to GMP, thereby decreasing the cGMP levels in the cytoplasm of the outer segment of the photoreceptor cell. This decrease results in a closure of cGMP-gated ion channels (CNG alpha- and beta subunits) and a subsequent hyperpolarization of the photoreceptor outer segment membrane. This hyperpolarization is carried throughout the cell and it decreases the rate of release of neurotransmitter (glutamate) at the synapse of the photoreceptor with the inner retinal neurons, mainly the bipolar cells.

In order to be ready to receive subsequent light stimuli, the activated members of the phototransduction cascade must be rapidly turned off and the cGMP should be replenished. cGMP is replenished by the activity of guanylate cyclase (GC) which converts GTP to cGMP. The deactivation of the rhodopsin (RHO) molecule occurs through phosphorylation by rhodopsin kinase (RK) and subsequent binding of arrestin (SAG) to the phosphorylated rhodopsin. The deactivation of the active transducin-phosphodiesterase complex is aided by the protein RGS9 (regulator of G-protein signalling) which is a protein anchored to outer segment disk membranes by R9AP (regulator of G protein signaling 9-binding protein). RGS9 accelerates the rate of guanosine triphosphate hydrolysis of GTP-bound transducin. The activity of RGS9 allows the rapid (in less than a second) return of transducin to its resting state. Activated transducin can return to its resting state without RGS9/R9AP, but the process takes tens of seconds. During this time frame, the photoreceptor is relatively insensitive to further light activation.

Photoreceptors from individuals with a defect in RGS9/R9AP have a noticeable prolongation of transducin activity after light exposure. The patient's may have temporary reduced vision for seconds to minutes after an initial light stimulus and,

as a result, have difficulties viewing moving objects against a light background. This causes decreased VA or, after an initial bright flash like that used to elicit ERGs, a response to subsequent flashes that is decreased for a tens of seconds longer period than in a normal eye. Patients describe symptoms of photophobia, problems adjusting to sudden changes in luminance, and difficulties seeing moving objects, as are described in **chapter 4**. A later study has shown that a patient lacking functional RGS9 has an increased retinal sensitivity at low luminance levels[107]. This effect is likely to result from prolonged activity of PDE6. Patients lacking RGS9 also do not gain visual sensitivity with increasing light levels as is seen in normals[107]. RGS9's activity is regulated through intracellular changes in calcium which affects phosphorylation at RGS9 through activation of protein kinase C[108]. RGS9 itself may additionally directly inhibit retinal guanylate cyclase[109]. These interactive loops may thus provide a delicate feedback mechanism allowing constant retinal function at changing light levels.

References

1. Gire,A.I. *et al.* The Gly56Arg mutation in NR2E3 accounts for 1-2% of autosomal dominant retinitis pigmentosa. *Mol. Vis.* 13, 1970-1975 (2007).
2. Coppieters,F. *et al.* Recurrent mutation in the first zinc finger of the orphan nuclear receptor NR2E3 causes autosomal dominant retinitis pigmentosa. *Am. J. Hum. Genet.* 81, 147-157 (2007).
3. Fingert,J.H. *et al.* Association of a novel mutation in the retinol dehydrogenase 12 (RDH12) gene with autosomal dominant retinitis pigmentosa. *Arch. Ophthalmol.* 126, 1301-1307 (2008).
4. Chakarova,C.F. *et al.* Mutations in TOPORS cause autosomal dominant retinitis pigmentosa with perivascular retinal pigment epithelium atrophy. *Am. J. Hum. Genet.* 81, 1098-1103 (2007).
5. Abd El-Aziz,M.M. *et al.* EYS, encoding an ortholog of Drosophila spacemaker, is mutated in autosomal recessive retinitis pigmentosa. *Nat. Genet.* 40, 1285-1287 (2008).
6. Collin,R.W. *et al.* Identification of a 2 Mb Human Ortholog of Drosophila eyes shut/spacemaker that Is Mutated in Patients with Retinitis Pigmentosa. *Am. J. Hum. Genet.*(2008).
7. Barragan,I. *et al.* Linkage validation of RP25 Using the 10K genechip array and further refinement of the locus by new linked families. *Ann. Hum. Genet.* 72, 454-462 (2008).
8. Zangerl,B. *et al.* Identical mutation in a novel retinal gene causes progressive rod-cone degeneration in dogs and retinitis pigmentosa in humans. *Genomics* 88, 551-563 (2006).
9. Zhang,Q. *et al.* Severe retinitis pigmentosa mapped to 4p15 and associated with a novel mutation in the PROM1 gene. *Hum. Genet.* 122, 293-299 (2007).
10. Leber,T. Ueber Retinitis pigmentosa und angeborene Amaurose. Albrecht *von Graefes Arch. Ophthal.*1-25 (1869).
11. Maguire,A.M. *et al.* Safety and efficacy of gene transfer for Leber's congenital amaurosis. *N. Engl. J. Med.* 358, 2240-2248 (2008).
12. Bainbridge,J.W. *et al.* Effect of gene therapy on visual function in Leber's congenital amaurosis. *N. Engl. J. Med.* 358, 2231-2239 (2008).
13. Gorbatyuk,M.S., Pang,J.J., Thomas,J., Jr., Hauswirth,W.W., & Lewin,A.S. Knockdown of wild-type mouse rhodopsin using an AAV vectored ribozyme as part of an RNA replacement approach. *Mol. Vis.* 11, 648-656 (2005).
14. Gorbatyuk,M., Justilien,V., Liu,J., Hauswirth,W.W., & Lewin,A.S. Preservation of photoreceptor morphology and function in P23H rats using an allele independent ribozyme. *Exp. Eye Res.* 84, 44-52 (2007).
15. O'Reilly,M. *et al.* RNA interference-mediated suppression and replacement of human rhodopsin in vivo. *Am. J. Hum. Genet.* 81, 127-135 (2007).
16. Tessitore,A. *et al.* Preferential silencing of a common dominant rhodopsin mutation does not inhibit retinal degeneration in a transgenic model. *Mol. Ther.* 14, 692-699 (2006).
17. Shinohara,T., Mulhern,M.L., & Madson,C.J. Silencing gene therapy for mutant membrane, secretory, and lipid proteins in retinitis pigmentosa (RP). *Med. Hypotheses* 70, 378-380 (2008).
18. Tanner,G. *et al.* Therapeutic strategy to rescue mutation-induced exon skipping in rhodopsin by adaptation of U1 snRNA. *Hum. Mutat.* 30, 255-263 (2009).
19. van Deutekom,J.C. *et al.* Local dystrophin restoration with antisense oligonucleotide PRO051. *N. Engl. J. Med.* 357, 2677-2686 (2007).
20. Buch,P.K., Bainbridge,J.W., & Ali,R.R. AAV-mediated gene therapy for retinal disorders: from mouse to man. *Gene Ther.* 15, 849-857 (2008).
21. O'Reilly,M. *et al.* A transgenic mouse model for gene therapy of rhodopsin-linked Retinitis Pigmentosa. *Vision Res.* 48, 386-391 (2008).

22. Guerin,K. *et al.* Systemic aminoglycoside treatment in rodent models of retinitis pigmentosa. *Exp. Eye Res.* 87, 197-207 (2008).
23. Grayson,C. *et al.* In vitro analysis of aminoglycoside therapy for the Arg120stop nonsense mutation in RP2 patients. *J. Med. Genet.* 39, 62-67 (2002).
24. Welch,E.M. *et al.* PTC124 targets genetic disorders caused by nonsense mutations. *Nature* 447, 87-91 (2007).
25. Kerem,E. *et al.* Effectiveness of PTC124 treatment of cystic fibrosis caused by nonsense mutations: a prospective phase II trial. *Lancet* 372, 719-727 (2008).
26. MacLaren,R.E. *et al.* Retinal repair by transplantation of photoreceptor precursors. *Nature* 444, 203-207 (2006).
27. Bartsch,U. *et al.* Retinal cells integrate into the outer nuclear layer and differentiate into mature photoreceptors after subretinal transplantation into adult mice. *Exp. Eye Res.* 86, 691-700 (2008).
28. Radtke,N.D. *et al.* Vision improvement in retinal degeneration patients by implantation of retina together with retinal pigment epithelium. *Am. J. Ophthalmol.* 146, 172-182 (2008).
29. Bertschinger,D.R. *et al.* A review of in vivo animal studies in retinal prosthesis research. *Graefes Arch. Clin. Exp. Ophthalmol.* 246, 1505-1517 (2008).
30. BRUCKNER,R. Slit-lamp microscopy and ophthalmoscopy in rat and mouse.. *Doc. Ophthalmol.* 5-6, 452-554 (1951).
31. Farber,D.B. From mice to men: the cyclic GMP phosphodiesterase gene in vision and disease. The Proctor Lecture. *Invest Ophthalmol. Vis. Sci.* 36, 263-275 (1995).
32. Bowes,C. *et al.* Retinal degeneration in the rd mouse is caused by a defect in the beta subunit of rod cGMP-phosphodiesterase. *Nature* 347, 677-680 (1990).
33. Travis,G.H., Brennan,M.B., Danielson,P.E., Kozak,C.A., & Sutcliffe,J.G. Identification of a photoreceptor-specific mRNA encoded by the gene responsible for retinal degeneration slow (rds). *Nature* 338, 70-73 (1989).
34. Bourne,M.C., Campbell,D.A., & Tansley,K. HEREDITARY DEGENERATION OF THE RAT RETINA. *Br. J. Ophthalmol.* 22, 613-623 (1938).
35. Davidorf,F.H. *et al.* Pathogenesis of retinal dystrophy in the Royal College of Surgeons rat. *Ann. Ophthalmol.* 23, 87-94 (1991).
36. Noell,W.K., Walker,V.S., Kang,B.S., & Berman,S. Retinal damage by light in rats. *Invest Ophthalmol.* 5, 450-473 (1966).
37. Hafezi,F., Grimm,C., Simmen,B.C., Wenzel,A., & Reme,C.E. Molecular ophthalmology: an update on animal models for retinal degenerations and dystrophies. *Br. J. Ophthalmol.* 84, 922-927 (2000).
38. Chang,G.Q., Hao,Y., & Wong,F. Apoptosis: final common pathway of photoreceptor death in rd, rds, and rhodopsin mutant mice. *Neuron* 11, 595-605 (1993).
39. Travis,G.H. Mechanisms of cell death in the inherited retinal degenerations. *Am. J. Hum. Genet.* 62, 503-508 (1998).
40. Lohr,H.R., Kuntchithapautham,K., Sharma,A.K., & Rohrer,B. Multiple, parallel cellular suicide mechanisms participate in photoreceptor cell death. *Exp. Eye Res.* 83, 380-389 (2006).
41. Kunchithapautham,K. & Rohrer,B. Apoptosis and autophagy in photoreceptors exposed to oxidative stress. *Autophagy.* 3, 433-441 (2007).
42. Wenzel,A., Grimm,C., Samardzija,M., & Reme,C.E. Molecular mechanisms of light-induced photoreceptor apoptosis and neuroprotection for retinal degeneration. *Prog. Retin. Eye Res.* 24, 275-306 (2005).
43. Sieving,P.A. *et al.* Ciliary neurotrophic factor (CNTF) for human retinal degeneration: phase I trial of CNTF delivered by encapsulated cell intraocular implants. *Proc. Natl. Acad. Sci. U. S. A* 103, 3896-3901 (2006).

44. Beltran,W.A., Wen,R., Acland,G.M., & Aguirre,G.D. Intravitreal injection of ciliary neurotrophic factor (CNTF) causes peripheral remodeling and does not prevent photoreceptor loss in canine RPGR mutant retina. *Exp. Eye Res.* 84, 753-771 (2007).
45. Wilson,R.B., Kunchithapautham,K., & Rohrer,B. Paradoxical role of BDNF: BDNF+/- retinas are protected against light damage-mediated stress. *Invest Ophthalmol. Vis. Sci.* 48, 2877-2886 (2007).
46. Merin,S., Obolensky,A., Farber,M.D., & Chowers,I. A pilot study of topical treatment with an alpha2-agonist in patients with retinal dystrophies. *J. Ocul. Pharmacol. Ther.* 24, 80-86 (2008).
47. Chang,C.J., Cherng,C.H., Liou,W.S., & Liao,C.L. Minocycline partially inhibits caspase-3 activation and photoreceptor degeneration after photic injury. *Ophthalmic Res.* 37, 202-213 (2005).
48. Rohrer,B. *et al.* Multidestructive pathways triggered in photoreceptor cell death of the rd mouse as determined through gene expression profiling. *J. Biol. Chem.* 279, 41903-41910 (2004).
49. Sanz,M.M. *et al.* Significant photoreceptor rescue by treatment with a combination of antioxidants in an animal model for retinal degeneration. *Neuroscience* 145, 1120-1129 (2007).
50. Gupta,N., Brown,K.E., & Milam,A.H. Activated microglia in human retinitis pigmentosa, late-onset retinal degeneration, and age-related macular degeneration. *Exp. Eye Res.* 76, 463-471 (2003).
51. Sharma,A.K. & Rohrer,B. Calcium-induced calpain mediates apoptosis via caspase-3 in a mouse photoreceptor cell line. *J. Biol. Chem.* 279, 35564-35572 (2004).
52. Hao,W. *et al.* Evidence for two apoptotic pathways in light-induced retinal degeneration. *Nat. Genet.* 32, 254-260 (2002).
53. Wenzel,A. *et al.* Prevention of photoreceptor apoptosis by activation of the glucocorticoid receptor. *Invest Ophthalmol. Vis. Sci.* 42, 1653-1659 (2001).
54. Ni,Y.Q. *et al.* Neuroprotective effects of naloxone against light-induced photoreceptor degeneration through inhibiting retinal microglial activation. *Invest Ophthalmol. Vis. Sci.* 49, 2589-2598 (2008).
55. Autoimmunity in Retinitis Pigmentosa. 2009. Ref Type: Data File
56. Mukherjee,P.K., Marcheselli,V.L., Serhan,C.N., & Bazan,N.G. Neuroprotectin D1: a docosahexaenoic acid-derived docosatriene protects human retinal pigment epithelial cells from oxidative stress. *Proc. Natl. Acad. Sci. U. S. A* 101, 8491-8496 (2004).
57. Bazan,N.G. Cell survival matters: docosahexaenoic acid signaling, neuroprotection and photoreceptors. *Trends Neurosci.* 29, 263-271 (2006).
58. Illing,M.E., Rajan,R.S., Bence,N.F., & Kopito,R.R. A rhodopsin mutant linked to autosomal dominant retinitis pigmentosa is prone to aggregate and interacts with the ubiquitin proteasome system. *J. Biol. Chem.* 277, 34150-34160 (2002).
59. Noorwez,S.M. *et al.* Pharmacological chaperone-mediated in vivo folding and stabilization of the P23H-opsin mutant associated with autosomal dominant retinitis pigmentosa. *J. Biol. Chem.* 278, 14442-14450 (2003).
60. Vallazza-Deschamps,G. *et al.* Excessive activation of cyclic nucleotide-gated channels contributes to neuronal degeneration of photoreceptors. *Eur. J. Neurosci.* 22, 1013-1022 (2005).
61. Bush,R.A., Kononen,L., Machida,S., & Sieving,P.A. The effect of calcium channel blocker diltiazem on photoreceptor degeneration in the rhodopsin Pro213His rat. *Invest Ophthalmol. Vis. Sci.* 41, 2697-2701 (2000).
62. Pearce-Kelling,S.E. *et al.* Calcium channel blocker D-cis-diltiazem does not slow retinal degeneration in the PDE6B mutant rcd1 canine model of retinitis pigmentosa. *Mol. Vis.* 7, 42-47 (2001).

63. Pawlyk,B.S., Li,T., Scimeca,M.S., Sandberg,M.A., & Berson,E.L. Absence of photoreceptor rescue with D-cis-diltiazem in the rd mouse. *Invest Ophthalmol. Vis. Sci.* 43, 1912-1915 (2002).
64. Yamazaki,H. *et al.* Preservation of retinal morphology and functions in royal college surgeons rat by nilvadipine, a Ca(2+) antagonist. *Invest Ophthalmol. Vis. Sci.* 43, 919-926 (2002).
65. Takano,Y. *et al.* Study of drug effects of calcium channel blockers on retinal degeneration of rd mouse. *Biochem. Biophys. Res. Commun.* 313, 1015-1022 (2004).
66. Takeuchi,K., Nakazawa,M., & Mizukoshi,S. Systemic administration of nilvadipine delays photoreceptor degeneration of heterozygous retinal degeneration slow (rds) mouse. *Exp. Eye Res.* 86, 60-69 (2008).
67. Perkins,G.A., Ellisman,M.H., & Fox,D.A. Three-dimensional analysis of mouse rod and cone mitochondrial cristae architecture: bioenergetic and functional implications. *Mol. Vis.* 9, 60-73 (2003).
68. Johnson,J.E., Jr. *et al.* Spatiotemporal regulation of ATP and Ca2+ dynamics in vertebrate rod and cone ribbon synapses. *Mol. Vis.* 13, 887-919 (2007).
69. Carmody,R.J., McGowan,A.J., & Cotter,T.G. Reactive oxygen species as mediators of photoreceptor apoptosis in vitro. *Exp. Cell Res.* 248, 520-530 (1999).
70. Carmody,R.J. & Cotter,T.G. Signalling apoptosis: a radical approach. *Redox. Rep.* 6, 77-90 (2001).
71. Yu,D.Y. & Cringle,S.J. Retinal degeneration and local oxygen metabolism. *Exp. Eye Res.* 80, 745-751 (2005).
72. Vingolo,E.M., Rocco,M., Grenga,P., Salvatore,S., & Pelaia,P. Slowing the degenerative process, long lasting effect of hyperbaric oxygen therapy in retinitis pigmentosa. *Graefes Arch. Clin. Exp. Ophthalmol.* 246, 93-98 (2008).
73. Bahrami,H., Melia,M., & Dagnelie,G. Lutein supplementation in retinitis pigmentosa: PC-based vision assessment in a randomized double-masked placebo-controlled clinical trial NCT00029289. *BMC. Ophthalmol.* 6, 23 (2006).
74. OT-551 Antioxidant Eye Drops to Treat Geographic Atrophy in Age-Related Macular Degeneration. Http://clinicaltrials.gov - study identifier: NCT00306488. 2008. Ref Type: Unpublished Work
75. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. *Arch. Ophthalmol.* 119, 1417-1436 (2001).
76. SanGiovanni,J.P. *et al.* The relationship of dietary carotenoid and vitamin A, E, and C intake with age-related macular degeneration in a case-control study: AREDS Report No. 22. *Arch. Ophthalmol.* 125, 1225-1232 (2007).
77. Shen,J.K. *et al.* Oxidative damage in age-related macular degeneration. *Histol. Histopathol.* 22, 1301-1308 (2007).
78. Brinton,R.D. Estrogen regulation of glucose metabolism and mitochondrial function: therapeutic implications for prevention of Alzheimer's disease. *Adv. Drug Deliv. Rev.* 60, 1504-1511 (2008).
79. Dykens,J.A. *et al.* Photoreceptor preservation in the S334ter model of retinitis pigmentosa by a novel estradiol analog. *Biochem. Pharmacol.* 68, 1971-1984 (2004).
80. Brinton,R.D. The healthy cell bias of estrogen action: mitochondrial bioenergetics and neurological implications. *Trends Neurosci.* 31, 529-537 (2008).
81. Phillips,M.J. *et al.* Tauroursodeoxycholic acid preservation of photoreceptor structure and function in the rd10 mouse through postnatal day 30. *Invest Ophthalmol. Vis. Sci.* 49, 2148-2155 (2008).
82. Berson,E.L., Sandberg,M.A., Rosner,B., Birch,D.G., & Hanson,A.H. Natural course of retinitis pigmentosa over a three-year interval. *Am. J. Ophthalmol.* 99, 240-251 (1985).

83. Berson,E.L. *et al.* A randomized trial of vitamin A and vitamin E supplementation for retinitis pigmentosa. *Arch. Ophthalmol.* 111, 761-772 (1993).
84. Berson,E.L. *et al.* Vitamin A supplementation for retinitis pigmentosa. *Arch. Ophthalmol.* 111, 1456-1459 (1993).
85. Radu,R.A. *et al.* Reductions in serum vitamin A arrest accumulation of toxic retinal fluorophores: a potential therapy for treatment of lipofuscin-based retinal diseases. *Invest Ophthalmol. Vis. Sci.* 46, 4393-4401 (2005).
86. Radu,R.A. *et al.* Accelerated accumulation of lipofuscin pigments in the RPE of a mouse model for ABCA4-mediated retinal dystrophies following Vitamin A supplementation. *Invest Ophthalmol. Vis. Sci.* 49, 3821-3829 (2008).
87. Weng,J. *et al.* Insights into the function of Rim protein in photoreceptors and etiology of Stargardt's disease from the phenotype in abcr knockout mice. *Cell* 98, 13-23 (1999).
88. Sun,H. & Nathans,J. Mechanistic studies of ABCR, the ABC transporter in photoreceptor outer segments responsible for autosomal recessive Stargardt disease. *J. Bioenerg. Biomembr.* 33, 523-530 (2001).
89. Redmond,T.M. *et al.* Rpe65 is necessary for production of 11-cis-vitamin A in the retinal visual cycle. *Nat. Genet.* 20, 344-351 (1998).
90. Wenzel,A., Reme,C.E., Williams,T.P., Hafezi,F., & Grimm,C. The Rpe65 Leu450Met variation increases retinal resistance against light-induced degeneration by slowing rhodopsin regeneration. *J. Neurosci.* 21, 53-58 (2001).
91. Keller,C., Grimm,C., Wenzel,A., Hafezi,F., & Reme,C. Protective effect of halothane anesthesia on retinal light damage: inhibition of metabolic rhodopsin regeneration. *Invest Ophthalmol. Vis. Sci.* 42, 476-480 (2001).
92. Sieving,P.A. *et al.* Inhibition of the visual cycle in vivo by 13-cis retinoic acid protects from light damage and provides a mechanism for night blindness in isotretinoin therapy. *Proc. Natl. Acad. Sci. U. S. A* 98, 1835-1840 (2001).
93. Weisinger,H.S., Vingrys,A.J., & Sinclair,A.J. Effect of dietary n-3 deficiency on the electroretinogram in the guinea pig. *Ann. Nutr. Metab* 40, 91-98 (1996).
94. Hoffman,D.R. *et al.* A randomized, placebo-controlled clinical trial of docosahexaenoic acid supplementation for X-linked retinitis pigmentosa. *Am. J. Ophthalmol.* 137, 704-718 (2004).
95. Berson,E.L. *et al.* Clinical trial of docosahexaenoic acid in patients with retinitis pigmentosa receiving vitamin A treatment. *Arch. Ophthalmol.* 122, 1297-1305 (2004).
96. Berson,E.L. *et al.* Further evaluation of docosahexaenoic acid in patients with retinitis pigmentosa receiving vitamin A treatment: subgroup analyses. *Arch. Ophthalmol.* 122, 1306-1314 (2004).
97. Leveillard,T. *et al.* Identification and characterization of rod-derived cone viability factor. *Nat. Genet.* 36, 755-759 (2004).
98. Ripps,H. Cell death in retinitis pigmentosa: gap junctions and the 'bystander' effect. *Exp. Eye Res.* 74, 327-336 (2002).
99. Shen,J. *et al.* Oxidative damage is a potential cause of cone cell death in retinitis pigmentosa. *J. Cell Physiol* 203, 457-464 (2005).
100. Komeima,K., Rogers,B.S., & Campochiaro,P.A. Antioxidants slow photoreceptor cell death in mouse models of retinitis pigmentosa. *J. Cell Physiol* 213, 809-815 (2007).
101. Komeima,K., Usui,S., Shen,J., Rogers,B.S., & Campochiaro,P.A. Blockade of neuronal nitric oxide synthase reduces cone cell death in a model of retinitis pigmentosa. *Free Radic. Biol. Med.* 45, 905-912 (2008).
102. Berson,E.L. Retinal degenerations: planning for the future. *Adv. Exp. Med. Biol.* 613, 21-35 (2008).
103. Mata,N.L., Radu,R.A., Clemmons,R.C., & Travis,G.H. Isomerization and oxidation of vitamin a in cone-dominant retinas: a novel pathway for visual-pigment regeneration in daylight. *Neuron* 36, 69-80 (2002).

104. Wolf,G. Transport of retinoids by the interphotoreceptor retinoid-binding protein. *Nutr. Rev.* 56, 156-158 (1998).
105. Mancil,R.M. *et al.* Improving nighttime mobility in persons with night blindness caused by retinitis pigmentosa: A comparison of two low-vision mobility devices. *J.. Rehabil. Res. Dev.* 42, 471-486 (2005).
106. Morrissette,D.L., Marmor,M.F., & Goodrich,G.L. An evaluation of night vision mobility aids. *Ophthalmology* 90, 1226-1230 (1983).
107. Stockman,A. *et al.* The loss of the PDE6 deactivating enzyme, RGS9, results in precocious light adaptation at low light levels. *J.. Vis.* 8, 10 (2008).
108. Sokal,I. *et al.* Identification of protein kinase C isozymes responsible for the phosphorylation of photoreceptor-specific RGS9-1 at Ser475. *J.. Biol. Chem.* 278, 8316-8325 (2003).
109. Bondarenko,V.A., Yu,H., Yamazaki,R.K., & Yamazaki,A. A novel role of RGS9: inhibition of retinal guanylyl cyclase. *Mol. Cell Biochem.* 230, 125-128 (2002).

Summary of results

Chapter 1 Retinitis pigmentosa (RP) is a hereditary degenerative disorder which primarily affects retinal rod photoreceptors and in later stages also the cone photoreceptors. The disease usually presents with night-blindness and gradual deterioration of the visual field starting from early adulthood, often ending in blindness at older age. The disease has a prevalence of 1 in 4000 and considers a major cause of visual impairment under the age of 60. Chapter 1 describes the whole spectrum of the retinitis pigmentosa with its symptoms, clinical findings, disease course, inheritance patterns, nutritional interventions and future perspectives. Chapter 1 furthermore summarizes the multiple genes associated with the development of retinitis pigmentosa, together with their respective prevalence and affected biochemical pathways. So far, over 45 genes have been identified, together accounting for about 60-70% of all patients.

The discovery of these multiple genes has led to an increasing knowledge of the retinal function, but the genetic heterogeneity still makes it a complex disease with respect to the understanding of common underlying processes and it explains the lack of curative therapy. The identification of novel genes causing RP or other hereditary retinal diseases, as well as factors that influence the varying severity of disease remain important in order to extend our knowledge on retinal function, and may give us clues with respect to the development of interventional strategies.

Chapter 2 describes the identification of a novel gene defect that causes recessive retinitis pigmentosa using a novel technique. Recessive RP is often caused by nonsense mutations that lead to low mRNA (messenger ribonucleic acid) levels as a result of nonsense-mediated decay. Some RP genes are expressed at detectable levels in leukocytes as well as the retina. Since retinal tissue is not readily available from living patients, we created lymphoblast cell lines of 13 unrelated RP families and 4 controls and isolated mRNA that was hybridized to the Affymetrix gene-chip "U133Plus 2.0" containing probe sets for over 47,000 human mRNAs. The comparison of hybridization intensities of individual transcripts revealed one index patient who had a highly significant reduction in hybridization measured by three probe sets that detected transcripts from the gene encoding the beta subunit of Nicotinamide adenine dinucleotide (NAD)-specific isocitrate dehydrogenase (*IDH3B*), an enzyme in the Krebs cycle. DNA (Deoxyribonucleic acid) sequencing of the *IDH3B* gene in the index patient and her affected brother revealed that both were homozygous for a 1-basepair deletion (Ile197fs; c.589delA) leading to a frameshift and a premature stop codon that is likely to result in rapid mRNA degradation through nonsense-mediated decay. A subsequent search for *IDH3B* mutations in 546 additional, unrelated patients revealed a patient with simplex RP who was homozygous for the missense mutation Leu132Pro; c.395T>C.

Enzyme assays showed a substantial reduction of NAD-specific IDH activity in lymphoblasts from both index patients. NAD-IDH is believed to catalyze the oxidation of isocitrate to alpha-ketoglutarate in the citric acid cycle, and is considered a fundamental enzyme. It is therefore surprising that our patients were otherwise healthy except for their RP. Nicotinamide adenine dinucleotide phosphate (NADP)-specific isocitrate dehydrogenase (NADP-IDH, or IDH2) considers an enzyme that catalyzes the same reaction, and normal activities of the NADP-IDH reaction were measured in our affected individuals.

In conclusion, we hypothesized that mitochondrial NADP-IDH, rather than NAD-IDH, serves as the main catalyst for this reaction in the citric acid cycle outside the retina, and that the retina has a particular requirement for NAD-IDH. Furthermore, the discovery of a new defect in mitochondrial metabolism as a cause of RP enhances interest in the role of mitochondria in RP and other neurodegenerations. Another point of interest is that the method used in this study may be applicable to other recessive human genetic diseases.

Chapter 3 describes the hypothesis test that chromosomal telomere length (repetitive elements at the end of chromosomes) is associated with disease severity in RP. Great variation exists in the age of onset of symptoms and the severity of disease at a given age in patients with RP. Unravelling factors that explain the phenotypic variation in patients with the same genotype may provide us new insights on the cellular level, and more importantly may give us clues with respect to therapeutic interventions. The final process by which photoreceptors and other cells are lost in retinal degenerations is through apoptosis. Telomere length is associated with apoptosis. To test the potential association between telomere length and severity of RP we evaluated 122 RP patients with the same mutation (Pro23His rhodopsin) and stratified their retinal function according to their 30-Hz cone electroretinogram (ERG). From 15 patients with highest ERG amplitudes and 15 patients with the lowest ERG amplitudes we evaluated the telomere lengths in leukocytes by the quantitative real time polymerase chain reaction (qRT-PCR) method. Results revealed no difference in telomere lengths between the groups with high or low ERG amplitudes. In conclusion, we found no association between telomere length and severity of RP.

Chapter 4 is not actually about retinitis pigmentosa, but it describes a non-progressive hereditary retinal disease, bradyopsia, of which the clinical findings are very important to the general knowledge of retinal function. The typical prolonged response suppression on the electroretinogram (ERG) was first described in 1991. Patients experienced symptoms of photophobia, problems adjusting to

bright light, and difficulties seeing moving objects. The responsible gene defect considered a mutation in the RGS9 (regulator of G-protein signalling 9) or R9AP (RGS9-1 anchor protein) gene which was described in 2004. In our department we had six patients with bradyopsia who were evaluated between 1973 and 2004. This chapter describes the clinical patient findings that were retrospectively obtained with follow-up up to 30 years. Patients showed a consistency of their symptoms and ERG recordings, but an extreme variation of visual acuity between visits. The low to subnormal visual acuity typically increased with use of pinholes. Increasing background luminances had a detrimental effect on the visual acuity. No progressive changes were seen over time.

RGS9 is a photoreceptor outer segment protein, anchored to and stimulated by R9AP, and is involved in the deactivation of the 'turned-on' transducin-phosphodiestrase complex in the phototransduction cascade: It accelerates the rate of guanosine triphosphate hydrolysis by releasing phosphate. This accelerative mechanism allows the rapid (in less than a second) return of the photoreceptor cell to its resting state. The activated complex can return to its resting state without RGS9/R9AP, but the process takes tens of seconds. During this time frame, the photoreceptor is temporarily immune to light activation. An individual with a defect in RGS9/R9AP, therefore, is blinded for a considerable amount of time after an initial light stimulus, and, as a result, has difficulties viewing objects against a bright background. This causes decreased VA or, after an initial bright flash like that used in ERG measurements, a response to subsequent flashes that is decreased for a longer period than in a normal eye.

Chapter 5 As outlined in chapter 1, patients with RP usually experience night-blindness from late puberty or early adolescence. Independent night time activities may thus be impaired throughout most of the patient's life. In this chapter we describe a study that evaluates the use of night-vision goggles by RP patients. Outcome measurements were 1) the change in mobility as expressed by the number of unintended contacts with obstacles (hits) and the percentage of preferred walking speed (PPWS) on a route in three different night time situations: a darkened indoor corridor, a moderately lit outdoor residential area, and a well lit outdoor shopping area; and 2) subjective evaluation and change in independent activities as measured with a personal journal and questionnaires. Assessments were performed before and after a 5 week training period.

Results showed a considerable mean decrease of hits in both the darkened corridor and the moderately lit outdoor residential area with the use of night-vision goggles, and a considerable increase of PPWS in the moderately-lit residential area. In the

shopping area there was no impaired mobility, and the night-vision goggles had no additional value. Subjective scores revealed a good sense of orientation, feelings of safety and tranquillity and an increase in independent mobility when night-vision goggles were used.

Chapter 6 In order to evaluate the usefulness of the night-vision goggles in daily life, we followed eleven RP patients from the study described in chapter 5 who had purchased the instrument at the end of the study period. After one and two years of use, they were requested to fill-in two questionnaires regarding their use of the instrument. The subjects still indicated improved mobility at night time with the use of their goggles. Nine out of eleven subjects still used the instrument after two years and reported an increase in their sense of independence and their potential for activities. Seven of them even used the instrument at least twice a week, indicating that the device could thus be implemented in the daily lives of our patients.

Chapter 7 provides an overview of this thesis and in particular future perspectives for RP. The multiple genes associated with RP are involved in different structural, functional and signalling retinal pathways, or may even consider defects in genes with non-retinal-specific general body functions. Interventional therapies may be focussed on different levels in the development of RP, ranging from the initial gene defect, the subsequent intermediate pathways that trigger photoreceptor rod cell death, factors that stimulate cell survival, or factors that cause the secondary loss of cone photoreceptors. Although developments with the highest chance of success for an ultimate cure, namely gene therapy that handles the initial defect, are rapidly evolving, many drawbacks should be encountered, and a rationale for the situation in RP is given in this chapter. Experiences from previous animal and human studies that concern different types of interventional strategies for RP are outlined in this chapter. The main suggestions for further explorations are aimed at the preservation of the secondarily affected cones and include, among others, antioxidant therapy, which may amplify the effect of vitamin A therapy and omega-3 rich fish diet.

Nederlandse samenvatting

Hoofdstuk 1. Retinitis pigmentosa (RP) is een erfelijke in ernst toenemende netvlies aandoening waarbij in eerste instantie de staafjes in de retina worden aangedaan en in latere stadia ook de kegeltjes. De aandoening presenteert zich over het algemeen met nachtblindheid en geleidelijke beperking van het gezichtsveld. Dit treedt vaak op vanaf de late pubertijd of vroege volwassenheid, en leidt in vele gevallen uiteindelijk tot totale blindheid. De ziekte komt voor bij 1 op de 4000 mensen en is een belangrijke oorzaak voor visuele handicap onder de leeftijd van 60 jaar. Hoofdstuk 1 beschrijft het hele spectrum van RP met de symptomen, klinische bevindingen, beloop, erfelijkheid, nutritionele interventies en toekomstperspectieven. Hoofdstuk 1 beschrijft tevens de multipele genen die geassocieerd zijn met de ontwikkeling van RP, samen met hun prevalenties en betrokken biochemische processen. Tot dusver zijn meer dan 45 genen ontdekt, welke in totaal ongeveer 60-70% van alle gevallen van RP verklaren.

De ontdekking van deze genen heeft geleid tot een toenemend inzicht in de functie van de retina. Echter, de genetische heterogeniteit maakt het een complexe ziekte met betrekking tot het begrip van alle onderliggende mechanismen en het verklaart het gebrek aan genezende behandelingsmogelijkheden. De ontdekking van nieuwe genen verantwoordelijk voor RP of andere erfelijke netvliesaandoeningen, alsook de identificatie van factoren die de wisselende ernst van de ziekte-expressie kunnen verklaren, zijn van belang voor de uitbreiding van onze kennis over processen in de retina, en kunnen ons aanknopingspunten bieden voor de ontwikkeling van therapeutische strategieën.

Hoofdstuk 2 beschrijft de ontdekking van een nieuw gen voor RP met gebruik van een nieuwe onderzoeksmethode. Recessief overgeërfde RP wordt vaak veroorzaakt door nonsense mutaties (mutaties met een vervroegd optredend stop codon) welke resulteren in lage mRNA (messenger ribonucleic acid) concentraties als gevolg van een nonsense-gemedieerd afbraak proces. Sommige genen geassocieerd met RP zijn ook detecteerbaar in lymfoblasten. Omdat retina weefsel nu eenmaal niet beschikbaar is van levende patiënten, creëerden we lymfoblast cellijnen van 13 RP families en 4 controle personen. Uit deze cellijnen werd mRNA geïsoleerd en aangebracht op een genchip (Affymetrix "U133Plus 2.0") welke meer dan 47,000 menselijke mRNA's kan detecteren. Alle mRNA niveaus van de verschillende families werden met elkaar vergeleken. Bij één familie werd een duidelijke afname van mRNA gezien dat codeerde voor het beta subunit van het Nicotinamide Adenine Dinucleotide (NAD)-afhankelijke isocitraat dehydrogenase (*IDH3B*), een enzym in de Krebs cycle (citroenzuur cyclus). In deze familie en 546 overige RP families werd vervolgens het DNA van dit gen geanalyseerd. Dit resulteerde in de identificatie van twee families met verschillende homozygote mutaties in het

IDH3B gen. Enzym testen werden verricht en toonden een duidelijke afname van NAD-afhankelijke IDH activiteit in lymfoblasten van deze beide families. NAD-afhankelijke IDH zou de derde stap in de citroenzuurcyclus katalyseren (oxidatie van isocitraat naar alpha-ketoglutaraat) en wordt beschouwd als een fundamenteel enzym. Het is daarom verrassend dat onze RP patiënten verder gezond waren. Er bestaat echter ook een ander enzym dat dezelfde reactie kan katalyseren, namelijk Nicotinamide Adenine Dinucleotide Phosphate (NADP)-afhankelijke isocitraat dehydrogenase (NADP-IDH, of *IDH2*). Van deze reactie werden normale waarden gemeten in onze aangedane patiënten.

Onze hypothese is dat buiten de retina het mitochondriale NADP-IDH, eerder dan NAD-IDH, dient als de hoofdkatalysator van de derde stap in de citroenzuur cyclus, en dat de retina een specifieke behoefte heeft aan NAD-IDH. Het in deze studie gevonden defect in het mitochondriale metabolisme als oorzaak voor RP vergroot de interesse voor de rol van mitochondria in RP en andere neurodegeneratieve aandoeningen. Een derde punt van belang van deze studie is dat de succesvolle toepassing van een nieuwe methode ter opsporing van recessieve genen ook gebruikt kan worden voor onderzoek naar andere recessief overgeërfde genetische aandoeningen.

Hoofdstuk 3 beschrijft de hypothese toets dat de telomeerlengte van chromosomen (herhaaldelijke elementen aan de uiteinden van chromosomen) geassocieerd is met de ernst van RP. Er bestaan forse verschillen in de ernst van RP en in de leeftijd waarop symptomen beginnen. De identificatie van factoren die de fenotypische variatie bij een identiek genotype verklaren, zouden ons nieuwe inzichten kunnen verschaffen in de pathologische processen op cellulair niveau, en belangrijker, zouden ons aanwijzingen kunnen geven voor therapeutische interventies. In retinale degeneraties gaan fotoreceptoren uiteindelijk vaak verloren door apoptose (= geprogrammeerde celdood). Telomeerlengte is geassocieerd met apoptose. Om de potentiele associatie tussen telomeerlengte en ernst van RP te testen, hebben wij uit een groep van 122 RP patiënten met de zelfde mutatie (Pro23His rhodopsine), 15 patiënten met de beste retina functie en 15 patienten met de slechtste retina functie (gemeten met 30-Hz kegel electroretinogram) geselecteerd, en hun leukocyt telomeerlengte gemeten met behulp van de 'quantitatieve real time polymerase chain reaction' (qRT-PCR) methode. De resultaten lieten een gelijke gemiddelde telomeerlengte zien tussen de groepen met hoge en lage ERG amplitudes. Concluderend vonden wij geen associatie tussen telomeerlengte en ernst van RP.

Hoofdstuk 4 gaat over bradyopsie, een niet in ernst toenemende erfelijke retinale aandoening waarvan de klinische bevindingen belangrijk zijn voor de algemene kennis over de functie van de retina. De bij de aandoening behorende typische verlengde suppressie van het elektroretinogram werd voor het eerst beschreven in 1991. Patiënten ervaren klachten van fotofobie, problemen met aanpassing aan veranderingen in lichtomstandigheden, en moeilijkheden met het herkennen van bewegende voorwerpen. Verantwoordelijk hiervoor is een mutatie in het RGS9 (regulator of G-protein signalling 9) of R9AP (RGS9-1 anchor protein) gen, welke voor het eerst werden beschreven in 2004. Op onze oogheelkunde afdeling hadden we totaal zes patiënten met bradyopsie met een poliklinische follow-up tussen 1973 en 2004. Hoofdstuk 4 beschrijft de bevindingen voor deze patiënten. Alle patiënten hadden consistente symptomen en afwijkingen op het electroretinogram, maar een grote variatie in de gemeten gezichtsscherpte bij verschillende polikliniekbezoeken. Opvallend was verder de verbetering van de gezichtsscherpte bij gebruik van de stenopeïsche opening. Verhoging van de achtergrondverlichting had een nadelig effect op de gezichtsscherpte. In de loop van de jaren werd geen daling van de gezichtsscherpte of verergering van de klachten en afwijkingen op het electroretinogram waargenomen. RGS9 is een enzym in de buitenste segmenten van fotoreceptoren, waar het verankerd is en gestimuleerd wordt door R9AP. RGS9 zorgt voor een snel (in minder dan 1 seconde) herstel van de fotoreceptor naar zijn rusttoestand, door een versnelde uitschakeling van het geactiveerde transducin-phosphodiesterase complex in de fototransductiecascade. Dit complex kan ook uitgeschakeld worden zonder de aanwezigheid van RGS9/ R9AP, maar dat duurt dan tientallen seconden. Gedurende deze periode is de fotoreceptor tijdelijk ongevoelig voor licht. Een persoon met een defect in RSGS9/ R9AP is daarom tijdelijk verblind na een sterke stimulatie met licht, en heeft als gevolg moeilijkheden met het zien van voorwerpen tegen een heldere achtergrond. Dit verklaart ook de verminderde gezichtsscherpte wanneer die gemeten wordt met een lichtprojector, en de toename in gezichtsscherpte met een stenopeïsche opening doordat de lichtinval op de retina afneemt. De tijdelijke ongevoeligheid voor licht verklaart ook de verlengde afwezige reactie op volgende lichtflitsen na een eerste heldere lichtflits zoals die wordt gebruikt bij metingen met het electroretinogram.

Hoofdstuk 5 Zoals is aangegeven in hoofdstuk 1, ervaren patiënten met RP veelal klachten van nachtblindheid vanaf de late pubertijd of vroege volwassenheid. Onafhankelijke avondactiviteiten kunnen gedurende het grootste deel van het leven van RP patiënten beperkt zijn. In dit hoofdstuk beschrijven we een studie waarin het gebruik van de nachtzichtbril door twintig RP patiënten wordt geëvalueerd. We bepaalden: 1) de verandering in mobiliteit uitgedrukt in het aantal onbedoelde

contacten met voorwerpen (botsingen) en de loopsnelheid als percentage van de persoonlijke voorkeurs loopsnelheid (relatieve loopsnelheid), gemeten op een route in drie verschillende avondsituaties: een donkere gang met voorwerpen, een matig verlichte woonbuurt, en een goed verlichte winkelbuurt; en 2) de subjectieve evaluatie en verandering in onafhankelijke activiteiten door middel van een persoonlijk dagboek en vragenlijsten. De metingen werden verricht voor en na een trainingsperiode van 5 weken. De resultaten lieten een duidelijke verbetering van de avondmobiliteit zien met het gebruik van de nachtzichtbril door een gemeten afname in botsingen in zowel de donkere gang met voorwerpen als de matig verlichte woonbuurt en een duidelijke toename van de relatieve loopsnelheid in de matig verlichte woonbuurt. In de goed verlichte winkelbuurt was er sprake van een goede mobiliteit zonder nachtzichtbril. Het hulpmiddel had hier dan ook geen toegevoegde waarde. Subjectieve evaluaties toonden een goed gevoel voor oriëntatie, gevoelens van veiligheid en rust, en een toename in zelfstandigheid in de avonduren met het gebruik van de nachtzichtbril.

Hoofdstuk 6 Voor een oordeel over het gebruik van de nachtzichtbril in het dagelijks leven van RP patiënten hebben we elf patiënten uit hoofdstuk 5 gevolgd die het hulpmiddel aan het einde van de studie periode hadden aan geschaft. Na één en na twee jaar gebruik, werden de patiënten verzocht om twee vragenlijsten met betrekking tot hun gebruik van de nachtzichtbril in te vullen. De deelnemers gaven een onveranderde verbetering van hun avondmobiliteit aan met het gebruik van de nachtzichtbril. Negen van de elf patiënten gebruikten het instrument nog na twee jaar en allen gaven een toegenomen gevoel van onafhankelijkheid aan, alsook toegenomen mogelijkheden om activiteiten te ondernemen. Zeven van de negen deelnemers gebruikten het hulpmiddel minstens twee keer per week. Dit geeft aan dat de nachtzichtbril dus toegepast kan worden in het dagelijks leven van onze patiënten.

Hoofdstuk 7 betreft de algemene discussie van dit proefschrift en in het bijzonder de toekomst perspectieven voor RP. De identificatie van oorzakelijke genen heeft geleid tot een snel toenemende kennis over de functie van de retina, maar geeft tegelijk vele vragen met betrekking tot de complexe pathofysiologische mechanismen. De multipele genen die geassocieerd zijn met RP zijn betrokken in verschillende structurele, functionele of aansturende processen in de retina, of betreffen zelfs defecten in genen met niet-retina-specifieke algemene lichaam functies. Interventietherapieën kunnen gericht zijn op verschillende niveau's in de ontwikkeling van RP, beginnend van het initiële gen defect, de daaropvolgende intermediaire processen die aanzetten tot de uiteindelijke celdood, factoren die overleving van de cel stimuleren, tot factoren die de oorzaak zijn van het secundaire

verlies van kegelfotoreceptoren. Hoewel de ontwikkelingen met de grootste kans op genezing, namelijk gentherapie, zich snel uitbreiden, moeten veel obstakels in acht worden genomen. De situatie omtrent RP en gentherapie wordt beschreven in dit hoofdstuk. Tevens worden de ervaringen van eerdere mens, dier, of overige laboratoriumstudies met betrekking tot verschillende soorten interventiestrategien voor RP beschreven. De voornaamste suggesties voor toekomstig onderzoek zijn gericht op het behoud van de secundair aangedane kegeltjes, en betreffen onder andere behandeling met antioxidanten, welke het eerder gemeten bescheiden effect van vitamine A therapie en omega-3-rijk vis dieet zou kunnen versterken.

Acknowledgement

Dankwoord

Dit proefschrift is tot stand gekomen met onderbroken periodes vanaf einde 2002 tot medio 2009, in combinatie met de opleiding tot oogarts. De projecten gedurende het promotietraject waren nogal verschillend, in de zin dat ik enerzijds wandelroutes met mijn deelnemers in de donkere avonduren in Groningen liep, en ik mij anderzijds met DNA onderzoek heb bezig gehouden te Boston, VS. Voor deze uiteenlopende studies heb ik gedurende de hele periode met veel verschillende mensen samengewerkt. Het was een prettige samenwerking en zonder hen was dit alles nooit tot stand gekomen. Bij deze wil ik een aantal mensen graag persoonlijk bedanken:

Als eerste mijn promotoren,
Professor Kooijman, beste Aart, jij vertrouwde mij het project van de nachtzichtbril toe. Vanaf het begin vond ik het een zeer ontspannen en gezellige samenwerking. Bedankt voor het delen van je uitgebreide kennis over de praktische uitvoering van een onderzoek, en voor de ruime vrijheid die je mij hierin gaf. Tevens bedankt dat jij mij vervolgens in contact hebt gebracht met Professor Dryja wat het mogelijk heeft gemaakt om onderzoek in Boston te doen.

Professor Hooymans, beste Anneke, mijn opleider en tevens promotor. Ik weet dat het niet gebruikelijk is om er gedurende de opleiding een jaar tussen uit te gaan. Ik heb onder andere hierdoor het hele opleidingsschema door de war gehaald. Mijn hartelijke dank dat je open stond voor het plan om een jaar onderzoek te doen in Boston en voor je vetrouwen en continue enthousiasme om dit in een promotietraject voort te zetten.

Professor Dryja, dear Ted, I am honored that you want to be my promoter and that you are willing to travel all the way to Groningen to be present at the defend of this thesis. You have been special in the fact that you hired me for one year to work in your lab despite my lack of knowledge in genetics. I enjoyed working in your lab including the weekly lab meetings, and on occasion a drink. You have been inspiring and you have been generous in the opportunities you gave me. Thank you for that and thank you for the freedom you gave me to choose some of my own projects. Sometimes you were worried that I did not enjoy the social life enough since I could stay in the lab quite late, but don't worry, there have been many hours in the lab, but I've also had a great time living in the city of Boston.

Bart Melis-Dankers, Frank Jorritsma, en Han Neve, bedankt voor jullie ideeën en hulp met betrekking tot het project van de nachtzichtbril en ook zeer belangrijk, dank voor jullie werk omtrent het vervolgtraject tezamen met D.Brouwer en Anne Vrijling, wat uiteindelijk heeft geleid tot de implementatie van de nachtzichtbril.

Bart, ik kan altijd genieten van de conversaties met jou en van de manier waarop je presentaties geeft, daar kan menigeen wat van leren. Verder wil ik de overige mensen van diverse instellingen in Nederland van Visio, Bartimeus, en Sensis bedanken voor hun hulp bij de uitvoering van het project: Herman Talsma, Frank Hoeben, Frans Riemslag, Peter Kerkhofs, Nadine Nauman, Wim van Damme en de mobiliteitstrainers: Roland van Grinsven, Thea Grotenhuis, Melissa Moll, José Packbier, Arianne Tange, Maren Tinselboer, Monique Mul, Cathelijne Kamp, José de Nijs, Greta Weber, Maaike Swennenhuis, Betsie Hollak, en Louise de Groot.

Klaas-Jan Bruin, het is inmiddels al weer een tijdje geleden, bedankt voor je hulp bij de statistische analyses. Jan-Willem Pott, bedankt voor de prettige samenwerking van oa het bradyopsie artikel, en de selectie van patienten met ERG afwijkingen. Ella, Fenna en Stella, bedankt voor jullie algemene hulp.

Professor Berson, I admire your tremendous work and your unexhaustive knowledge on retinitis pigmentosa. Thank you for your collaboration.
Dear Terri, what would I have done without you! You have not only teached me everyting in the lab, but you also knew a lot about basic science. I have very much enjoyed our endless conversations. Thank you for all your input and for being my friend. Geetha, I have enjoyed working with you and learned a lot about the live of an American college student. Meredith, you have been very kind. Thank you for your help, especially at the end of my stay and shortly thereafter. Nasim, Kristen, thank you for your grateful work on patient contacts. Marion, Linda, thank you for your general help. Dr. M. A. Sandberg, Dr. I de Vivo, Dr. R. F. Colman, Mayura Dange, and Pim van der Harst thank you for your collaboration.

Gedurende deze periode heb ik echter het grootste deel van mijn tijd doorgebracht bij de opleiding oogheelkunde waarmee ik over een aantal maanden klaar zal zijn. Mijn collega (oud) arts-assistenten: Carolien, Tanja, Angela, Kochoi, Lonneke, Govert, Rogier, Nicole, Karen, Remco, Esther, Suzan, Dirk, Theo, Danna, Annemiek, Eylem, Marieke, The Anh, Rosemarie, Stijn, Mathijs, bedankt voor jullie gezelligheid en fijne samenwerking op en buiten de polikliniek. Hiernaast wil ik ook alle stafleden, secretaresses, en overige medewerkers van de oogheelkundige polikliniek te Groningen bedanken voor de de prettige tijd op de werkvloer.

Alle deelnemers aan het onderzoek, mijn hartelijke dank voor jullie medewerking.

Mijn paranimfen Marjanneke en Danna; Marjanneke, wij kennen elkaar ongeveer vanaf het begin van dit promotietraject. Het is nu al jaren bijna een vaste prik

dat wij op woensdag op weg naar onze tennisles de gebeurtenissen van de week doornemen. Ik hoop dit nog lang vol te kunnen houden. Bedankt voor je luisterend oor, je interesse en je gezelligheid.
Danna, van alle collega's ben jij het meeste op de hoogte van mijn bezigheden ten behoeve van dit proefschrift. Ik dank je voor je oprechte interesse en je bereidheid je te willen inleven in een onderwerp waar je zelf weliswaar niet in thuis bent, maar waarmee je mij de kans wilt geven om er eens over te discussieren.

Lieve ouders, jullie zijn er altijd voor mij geweest, en jullie hebben mij gemaakt tot de persoon die ik nu ben. Bedankt dat jullie het voor mij mogelijk hebben gemaakt om zorgeloos te kunnen studeren en dat jullie mij onvoorwaardelijk steunden in de beslissingen die ik nam. Ook nu nog staan jullie altijd voor me klaar, met alle liefde laten jullie je kleindochter komen logeren zodat ik mijn dingen kan blijven doen. Verder wil ik tevens mijn schoonouders, Björn, Alexandra, Matthy, Michiel en Daniel bedanken voor hun oprechte interesse in mijn onderzoek.

Lieve Folkert, jij bent zo veel voor mij. Met jouw onvermoeibare wetenschappelijke activiteiten ben jij een bron van inspiratie, tegelijkertijd geef je mij ook rust door samen te genieten van de andere zaken in het leven. Met onze gedeelde interesse voor de wetenschap, hebben we het ondanks de verschillende vakgebieden voor elkaar gekregen om 1 studie gezamenlijk te publiceren (hoofdstuk 3, dit proefschrift). Ik dank je voor al je onvoorwaardelijke steun en liefde waarmee je het voor voor mij mogelijk hebt gemaakt om mijn proefschrift af te kunnen ronden, te meer omdat je het zelf ook altijd heel druk hebt. In deze periode hebben we samen een geweldige dochter, Babette, gekregen. In vele opzichten is zij echt een dochter van haar vader. Als alles goed gaat krijgen we er in augustus nog een kindje bij. Ik geniet van jou, van ons gezin en van ons immer volgeplande leventje. Ik hou van je.

Lieve Babette, je bent nog maar zo'n klein meisje, maar je bent al een hele persoonlijkheid. Je bent amper 1 jaar oud maar je lijkt precies te weten wat je wilt. Ik kijk er naar uit om jou op te zien groeien en iedere dag weer opnieuw van jou te mogen genieten. Het is een feest dat jij net als je vader en moeder in de weekenden kan uitslapen en daarvoor ben ik je zeer dankbaar.

Curriculum Vitae

Dyonne Hartong was born in Naarden, the Netherlands in 1977 and lived there her entire youth. She completed her secondary school at the Sint Vitus College in Bussum in 1995. That year she started her medical studies at the University of Groningen, and joined the student sorority R.K.S.V. Albertus Magnus. For her scientific program in the fourth year she studied the survival of rabbit retina in a tissue culture system at the University of Lund, Sweden under the supervision of dr. A. Bruun. In 2000 she started her internships which were mainly followed at the Medisch Spectrum Twente, Enschede, the Netherlands. She followed a complementary surgery internship at the department of Surgery, Hassanuddin University, Ujung Pandang (Makassar), Sulawesi, Indonesia, under the supervision of Prof. Dr. Chairuddin Rasjad, FICS. She did her complementary ophthalmology internship at the department of ophthalmology at the Vrije Universiteit Amsterdam under the supervision of drs. W.A.E.J. de Vries-Knoppert. After obtaining her medical degree in februari 2002, she temporarily worked in the function of medical officer in a municipal job project in Amsterdam. At the end of 2002 she started as a research fellow studying the role of night vision goggles for retinitis pigmentosa patients at the University of Groningen under the supervision of Prof. Dr. Kooijman. Since august 2003 she is in training for ophthalmologist under Prof. Dr. J.M.M. Hooymans at the department of ophthalmology, University Medical Center Groningen. From July 2005 to July 2006 she worked as a research fellow on retinal genetics at the Ocular Molecular Genetics Institute, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA, USA.