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Retinitis Pigmentosa in Northern Sweden – From Gene to Treatment

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1. Introduction

Blindness is a loss of vision resulting in the inability to continue with a normal lifestyle. The prevalence of blindness varies from country to country and from region to region within the same country. An average estimate of the number of blind people in industrialized countries is 1–2 per 2000, compared to 5–10 per 1000 in developing countries. According to World Health Organisation estimates, there are between 27 and 35 million blind people in the world today.

Retinal dystrophies and degenerations represent a heterogeneous group of disorders affecting the function of the retina. They are characterized by degeneration of photoreceptors or adjacent cells such as retinal pigment epithelium or Müller cells. Retinitis pigmentosa (RP), hereditary maculopathies, and age-related macular degeneration (AMD) are representative of these diseases. RP as well as macular degeneration can be part of syndromes with symptoms from other organs or organ systems, for example, Usher syndrome with RP and deafness. More than 190 loci for different retinal diseases have been localized, and 140 genes have been identified (http://www.sph.uth.tmc.edu/Retnet/).

In northern Sweden the presence of all hereditary disorders is higher than in the southern part of the country. Explanations offered include a low migration rate in the sixteenth to nineteenth centuries and a certain number of marriages among relatives, which influenced the presence of autosomal recessive disorders. Furthermore, a disposition to a genetic abnormality in a geographically restricted area gave rise to a 'founder' effect, which influenced the presence of autosomal dominant disorders. These factors are taken into consideration in studies of all hereditary disorders, including those affecting vision.

2. Retinitis pigmentosa

Retinitis pigmentosa is a genetically and clinically heterogeneous group of hereditary retinopathies characterized by a degeneration of photoreceptors (rods primary) with a progressive loss of peripheral vision. This leads to night blindness, 'tunnel vision', and eventually, complete blindness. Typical signs of the disease, except for night blindness and progressive loss of the peripheral visual field, are typical pigment deposition in the retina, attenuation of the retinal blood vessels, and optic disc pallor. The diagnosis is confirmed by an abnormal or extinguished electroretinogram (ERG). RP can be nonsyndromic, syndromic,

or systemic, when multiple tissues are affected. Nonsyndromic RP can be inherited in autosomal dominant (15–25%), autosomal recessive (5–20%), and X-linked (5–15%) manners (Daiger & Pagon, 2000). However, an inheritance pattern is still unknown in many cases. So-called simplex usually represents an isolated case without any family history. Due to different degrees of penetrance and expressivity of RP genes, the phenotype can vary between families and even within the same family (Shintani et al., 2009).

The prevalence in the United States and Europe is approximately 1/3500 to 1/4000, and the disorder is the most common cause of blindness among young adults. In Denmark the lifetime risk of developing RP is 1/2500 (Haim et al., 2002), and in Sweden it is 1/2000 (Burstedt et al., 1999). Similar frequencies can be expected in other populations, but they have not been well documented. Frequency of RP in certain isolated or consanguineous populations might be higher due to mutations in particular genes (Daiger & Pagon, 2000). Among known loci and genes there are 57 identified for autosomal dominant RP and autosomal recessive RP. Many genes associated with RP encode proteins functioning as photoreceptor transcription factors; others are involved in phototransduction and the visual cycle or photoreceptor structure (Phelan & Bok, 2000; Fig. 1). Despite many retinal diseases having been mapped to specific chromosomal regions, the genes and their functions still have not been identified in all cases.

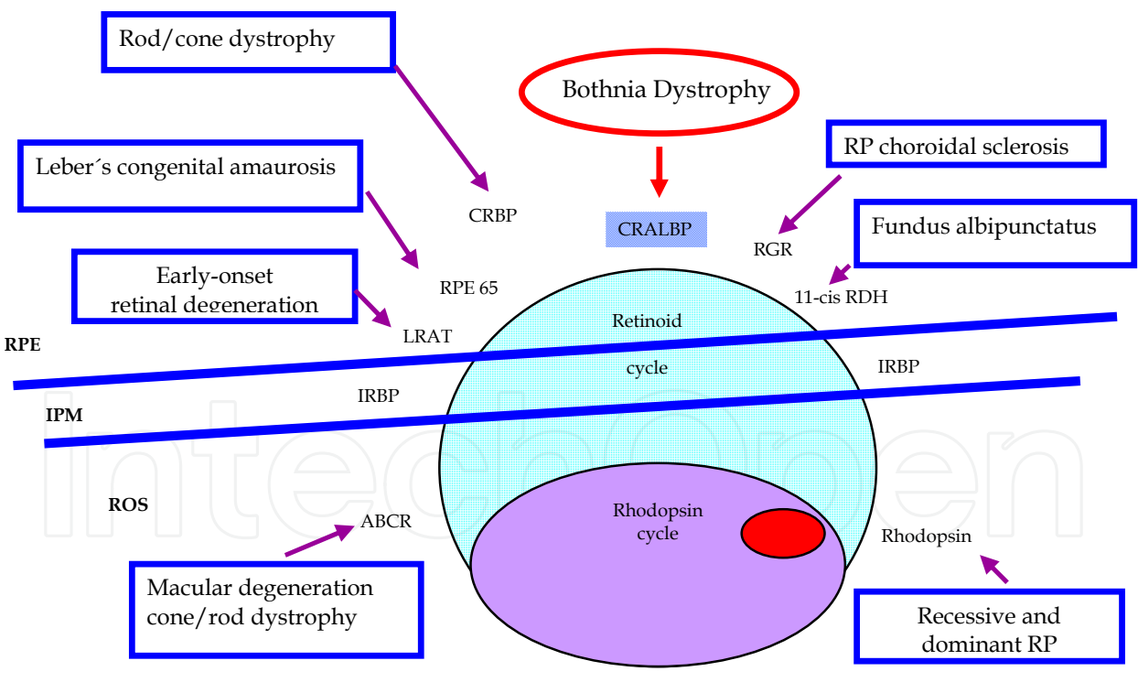

Fig. 1. Visual cycle proteins cause retinal degenerations. CRBP = cellular retinol binding protein, RPE65 = retinal pigment epithelium-specific protein 65kDa, LRAT = lecithin retinol acyltransferase, IRBP = interphotoreceptor retinoid-binding protein, RPE = retinal pigment epithelium, IPM = interphotoreceptor matrix, ROS = rod outer segment, ABCR = retina-specific ATP-binding cassette transporter, CRALBP = retinaldehyde-binding protein 1, RGR = retinal G protein coupled receptor, 11-cis RDH = retinol dehydrogenase 5 (11-cis/9-cis).

In Västerbotten County of northern Sweden 160 RP cases were identified and genetic defects were found in 66%, representing 79 patients with autosomal recessive RP of Bothnia type and 27 patients with autosomal dominant RP. However, genetic mechanisms of RP are still unknown in patients from other counties of northern Sweden. In this chapter we will focus on a disease with characteristic phenotype common in northern Sweden form of autosomal recessive RP, Bothnia dystrophy.

2.1 Autosomal recessive RP, Bothnia Dystrophy (BD)

Examination of medical records for patients with RP in Västerbotten County in northern Sweden has shown an accumulation of cases with a unique phenotype of RP called Bothnia dystrophy. Bothnia is the region in northern Sweden west of the Gulf of Bothnia, historically known as Bothnia Occidentalis (Fig. 2). Affected individuals showed night blindness with onset in early childhood, retinitis punctata albescens (RPA) at some stage of the disease, macular degeneration, and markedly elevated dark adaptation (DA) thresholds.

Fig. 2. The map shows the location of Västerbotten County in Scandinavia.

2.1.1 Genetic cause of BD

Initially, twenty patients from seven families originating from the same geographic area were included in the linkage analysis study aimed at identifying a disease-causing gene (Burstedt et al., 1999). All affected individuals had a nonsyndromic type of retinal degeneration inherited in an autosomal recessive way (Fig. 3). By statistical two-point lod (logarithm [base 10] of odds) score analysis, which is used to determine the linkage between trait and a chromosome marker, BD was mapped between the markers D15S526 and FES on chromosome 15q26.1. The D15S116, located near the *RLBP1* gene showed a maximum lod score of 7.79. Since lod score greater than 3 is considered evidence for linkage, the *RLBP1* gene was a strong candidate to cause the BD. Mutation analysis showed that all patients were homozygous for a cytosine (C) to thymine (T) change in exon 7 of the *RLBP1* gene, resulting in a substitution of a conservative arginine to tryptophan at position 234

(c.700C>T, p.R234W) in encoded cellular retinaldehyde-binding protein (Burstedt et al., 1999). No homozygotes for the c.700C>T mutation were found among 33 unaffected control subjects who underwent ophthalmologic examinations or 92 anonymous blood donors.

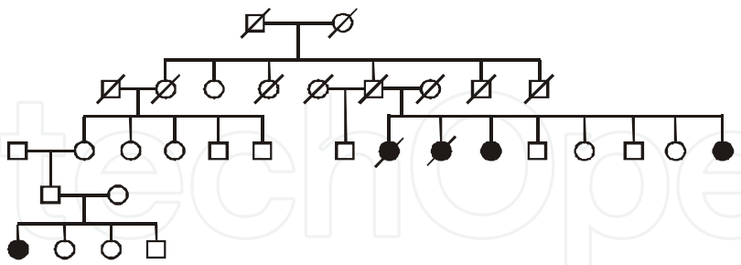

Fig. 3. A pedigree demonstrating autosomal recessive inheritance of BD in one of the families used for linkage analysis study.

2.1.2 Compound heterozygosity in BD

Sixty-nine BD cases homozygous for the c.700C>T were identified amongst patients with retinal dystrophies. In addition, 10 patients with similar to BD phenotype were heterozygous. Further screening for known mutations causing autosomal recessive RP revealed a second *RLBP1* mutation, a thymine (T) to adenine (A) change, resulting in a substitution of a methionine to lysine, c.677 T>A, p.M226K (Köhn et al., 2008). R234W and M226K were shown to be allelic and the patients were compound heterozygotes, c.[677T>A]+[700C>T] (Fig. 4).

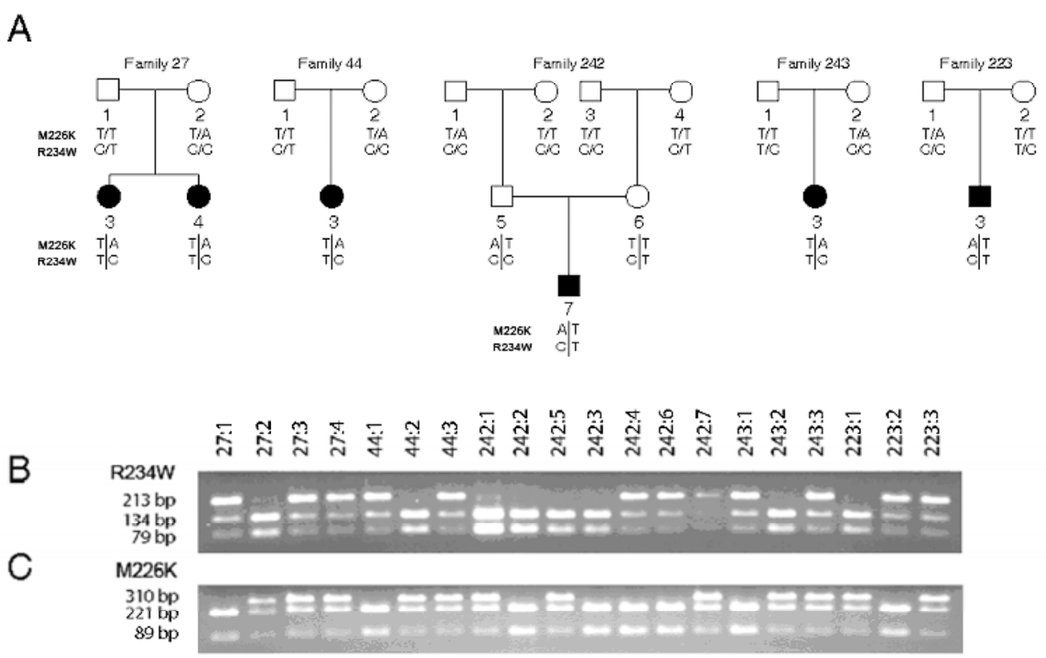

Fig. 4. Pedigrees of compound heterozygotes (A) and segregation analysis of *RLBP1* c.700C>T (B) and c.677T>A (C) performed by polymerase chain reaction and restriction fragment length polymorphism. (Figure published in Invest Ophthal Vis Sci, 2008;49(7):3172–3177).

Frequency of c.677 T>A allele in a matched control population was 0.0021. Among RP patients from northern Sweden two were homozygotes for p.M226K mutation. Based on allele frequency of R234W (3/250) and M226K (1/466), we believe that R234W was the first mutation to appear in northern Sweden, resulting in expected disease incidence of 1.5 per 10,000 in a population of 257,000 inhabitants.

Notably, one *RLBP1* compound heterozygote BD patient tested for 848 mutations in 29 genes causing autosomal dominant RP was shown to be a carrier of a mutation in carbonic anhydrase IV (CAIV), known to be associated with autosomal dominant retinitis pigmentosa RP17 (Rebello et al., 2004; Yang et al., 2005). Presence of this sequence variant in 6 out of 143 blood donors from a control Swedish population (4%) and phenotype undistinguishable from the other BD patients casts doubt on the pathogenic role of the CAIV (Köhn et al., 2008), though modifier genes switching off the mutant CAIV protein or other factors resisting its function in carriers of Swedish origin remain to be investigated.

2.1.3 *RLBP1* mutations in RP

To date, at least 12 mutations representing single nucleotide changes, in addition to small and one gross deletion have been reported (http://www.hgmd.cf.ac.uk). First homozygous *RLBP1* mutation was found in a consanguineous family of Indian origin and in one of consanguineous kindred from Saudi Arabia, both diagnosed with retinitis punctata albescens (Maw et al., 1997; Katsanis et al., 2001). Three additional mutations in the *RLBP1* gene were identified in the patients of European ancestry with recessively inherited RPA (Morimura et al., 1999), and in patients of Newfoundland origin with a severe rod cone dystrophy two splice junction mutations were detected (Eichers et al., 2002). The reported cases of retinal degeneration associated with *RLBP1* mutations were either homozygous or compound heterozygous (Demirci et al., 2004; Eichers et al., 2002; Fishman et al., 2004; Morimura et al., 1999; Nakamura et al., 2005). One of the compound heterozygotes, a Japanese patient with RPA, carried the c.700C>T mutation on one allele (Nakamura et al., 2005). Finally, changes involving single nucleotides are not the only type of mutation that affects the *RLBP1* gene. A large homozygous deletion was described in a RPA patient (Humbert et al., 2006).

2.1.4 Cellular retinaldehyde-binding protein (CRALBP)

RLBP1 gene mapped to chromosome 15q26 (Rosenfeld & Dryja, 1995) encodes the human cellular retinaldehyde-binding protein (CRALBP) expressing in outer epithelium of the iris, ciliary body pigment epithelium, cornea, optic nerve, pineal gland, Müller cells of the retina, and retinal pigment epithelium (RPE) (Bridges et al., 1987; Bunt-Milan & Saari, 1983; Eisenfeldt et al., 1985; Futterman & Saari, 1977; Saari et al., 1997; Sarthy, 1996). In the RPE, CRALBP functions as a carrier protein for endogenous retinoids, such as 11-cis-retinol, participating in the visual cycle. 11-cis-retinol can either be stored as an ester in the RPE or become oxidized to 11-cis-retinal by 11-cis-retinol dehydrogenase for visual pigment regeneration, and consecutively recycled back to the outer segment of photoreceptor cells of the retina (Saari, 1990). In vitro studies indicate that the presence of CRALBP diminishes the esterification and enhances oxidation of 11-cis-retinol (Saari et al., 1994).

A missense mutation of a conserved residue arginine at position 150 of CRALBP abolished binding to 11-cis-retinaldehyde. The mutation was shown to be associated with an atypical form of autosomal recessive RP in a small consanguineous Indian family (Maw et al., 1997).

We evaluated binding activities for two recombinant mutant proteins causing BD (Golovleva et al., 2003). M226K mutation completely abolished binding of the recombinant protein with 11-cis retinaldehyde, while the R234W, in contrast, increased binding activity of the recombinant CRALBP (Fig. 5). Double mutant M226K + R234W showed less solubility than the wild type rCRALBP (Köhn et al., 2008).

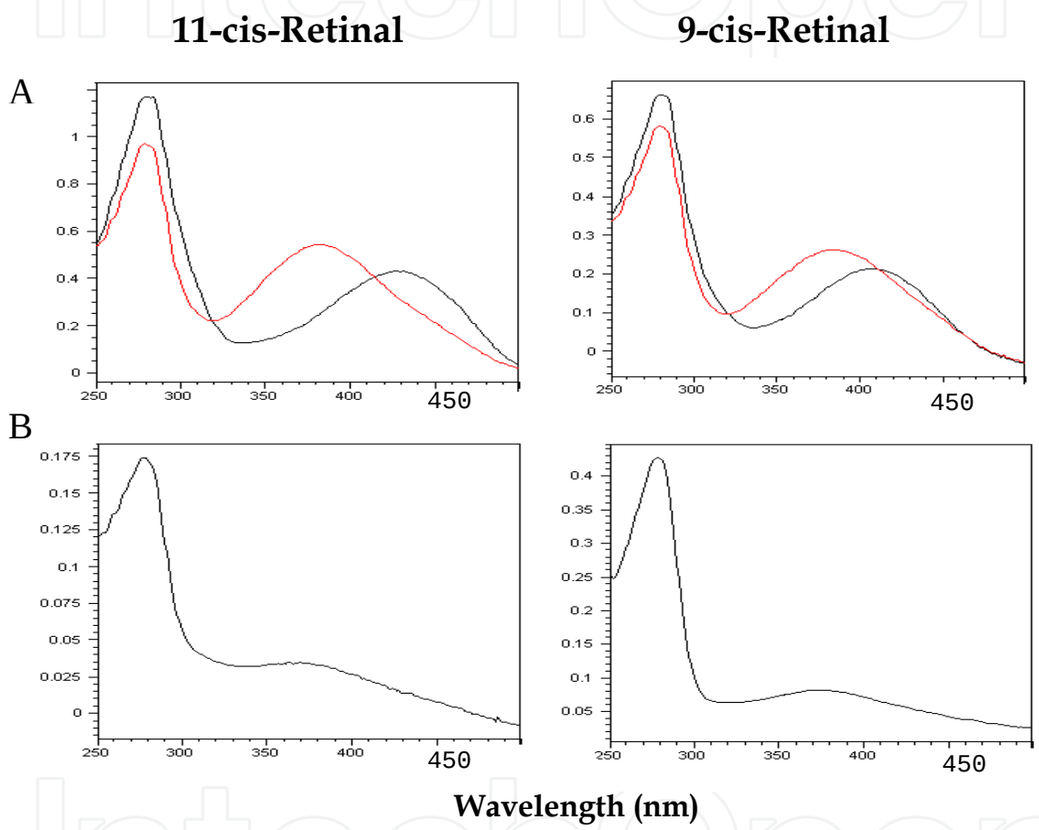

Fig. 5. Retinoid binding analysis of CRALBP mutants R234W (A) and M226K (B). UV-visible absorption spectra are shown before and after exposure to bleaching illumination following retinoid labelling with either 11-cis- or 9-cis-retinal. Spectra from wild-type rCRALBP (black line) and mutant R234W (red line) are indistinguishable and reflect stoichiometric binding of 11-cis- and 9-cis-retinal. The absorption spectra from mutant M226K show no chromophore absorbance at 425 nm or 400 nm, indicating no bound retinoid (Figure published in J Biol Chem, 2001;278:14,12397–12402).

We also analysed *RLBP1* promoter by sequencing 4kb in the upstream region in two BD compound heterozygotes. Seven single nucleotide polymorphisms (SNP) were identified; six had been reported previously and one was unique (cytosine to thymine change at position 2614, C2614T). Haplotype was constructed for BD patients. R234W and C2614T

were both associated with BD phenotype. In experiments with reporter gene expression, decrease of expression level was shown when using either the entire 'affected' promoter haplotype or only C2614T (Golovleva, personal communication).

Thus, M226K mutation resulted in loss of functional CRALBP, which is true even for R234W mutant, since gene expression was decreased due to sequence variant in promoter.

2.2 Phenotype of retinitis pigmentosa of Bothnia type

All 79 patients originating from Västerbotten County, with a population of 257,000 inhabitants, present the phenotype caused by loss of CRALBP function.

2.2.1 Clinical findings and effects of age

The BD phenotype is characterized by central and peripheral degeneration of the retina with a unique expression of retinitis pigmentosa. All BD patients from the north of Sweden being homozygous for either the c.700C>T (p.R234W) (n = 67) or for the c.677T>A (p.M226K) (n = 2), and compound heterozygous [c.677T>A]+[c.700C>T] (p.M226K+p.R234W) (n = 10), have a clinical expression of the disease with a progression of retinal degeneration. The progressive maculopathy in BD presents an overall decrease of the visual acuity (VA) with age, leading to legal blindness in early adulthood (Fig. 6; Burstedt et al., 2001).

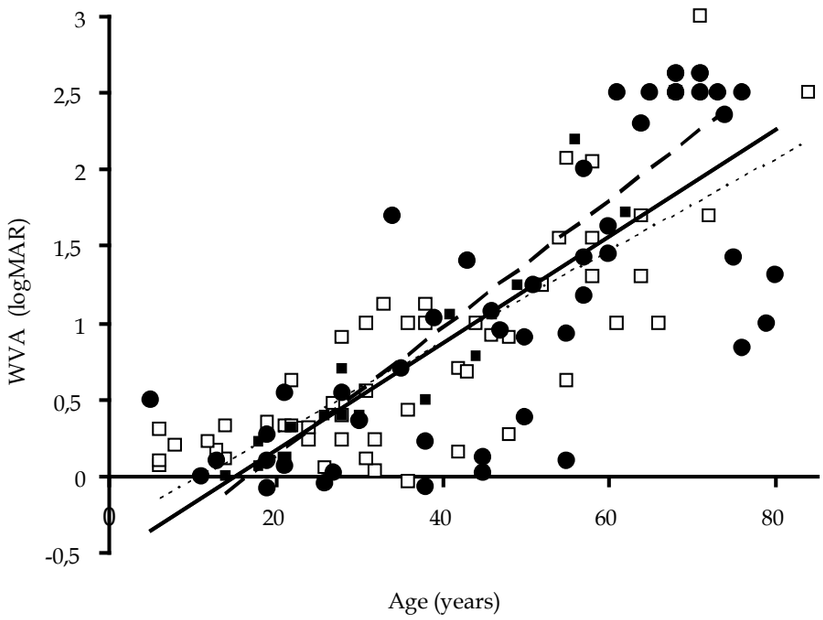

Fig. 6. Scatterplot of BD patients showing the relation of weighted distance logMAR visual acuity (WVA) with age. (●) Single observations of WVA vs. age in homozygotes c.700C>T (p.R234W); n = 49, (y = 0.035 × -0.53). (□) Retrospective recordings of WVA vs. age in compound heterozygotes [c.677T>A] + [c.700C>T] (p.M226K + p.R234W); n = 10 (y = 0.04 × −0.7), and homozygotes c.677T>A (p.M226K) (■); n = 2 (y = 0.03 × -0.32). Trendlines are drawn.

Notably, testing of monocular low-contrast VA using Sloan letter logarithmic translucent contrast charts (10% and 2.5%, Precision Vision®) allowed recording of measurable results, predominantly in the younger patients (Burstedt et al., 2005).

The retinal findings in BD patients show distinct maculopathy with central pigment deposits in the teens, and areolar maculopathy is observed in the younger adults (Burstedt et al., 1999, 2001, 2010). In the peripheral retina the pigmentations are similar to a 'salt and pepper' pattern and round retinal atrophies develop paracentrally and/or peripherally with age. The areolar atrophies are the most common peripheral findings in BD fundus as the degeneration progresses, though discrete pigmentations with an appearance similar to bone spicules may occasionally be found. No premature cataract of significance is observed in BD, and in advanced cases narrowing of the retinal vessels and pale optic disc are not typical findings (Fig. 7).

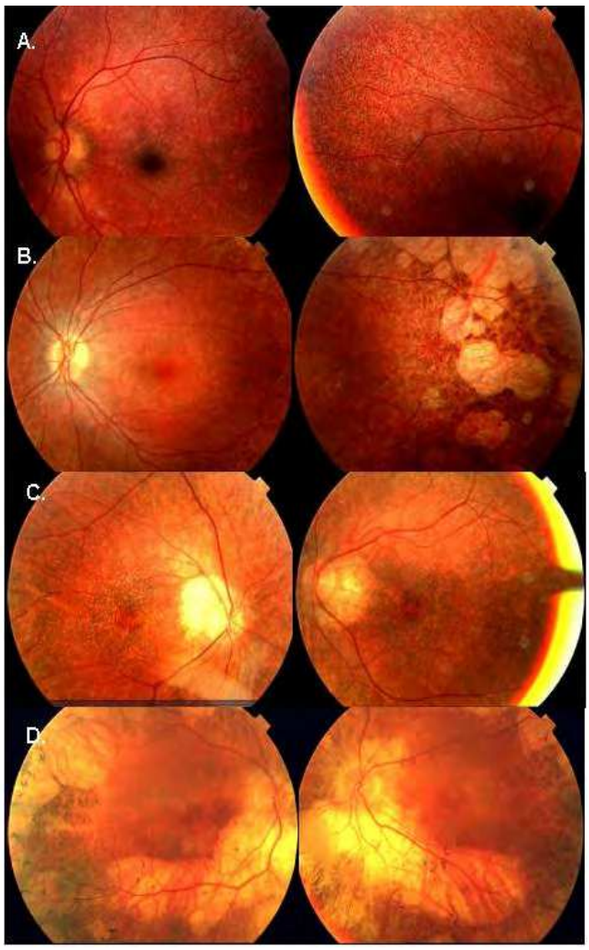

Fig. 7. Fundus photography. (A) 16-year-old girl, discrete central maculopathy and peripheral mottling, (WVA, weighted visual acuity, 0.00 logMAR). (B) 25-year-old woman with maculopathy and peripheral areolar atrophies (WVA 0.5 logMAR). (C) 41-year-old woman with central maculopathy and central RPA changes (WVA 0.3 logMAR). (D) 52-year-old woman with maculopathy, pigmentary changes (WVA 1.2 logMAR), and advanced retinal degeneration with retinal atrophies.

2.2.2 Morphological changes in BD found with ocular computed tomography

A generalized early decrease of the central foveal thickness (Ø 1 mm) and the inner ring of the retina (Ø 3 mm) are shown with optical coherence tomography (Burstedt et al., 2010). In the outer ring (Ø 6 mm), the generalized thinning is seen predominately in the inferior regions of the OCT measurement and a trend of more preserved areas of the retinal thickness of the superior and nasal regions are observed. The total macular volumes in all ages of BD patients are low compared with those of controls. Comparisons of macular thickness in each region of the BD patients and age-matched controls are presented in Fig. 8.

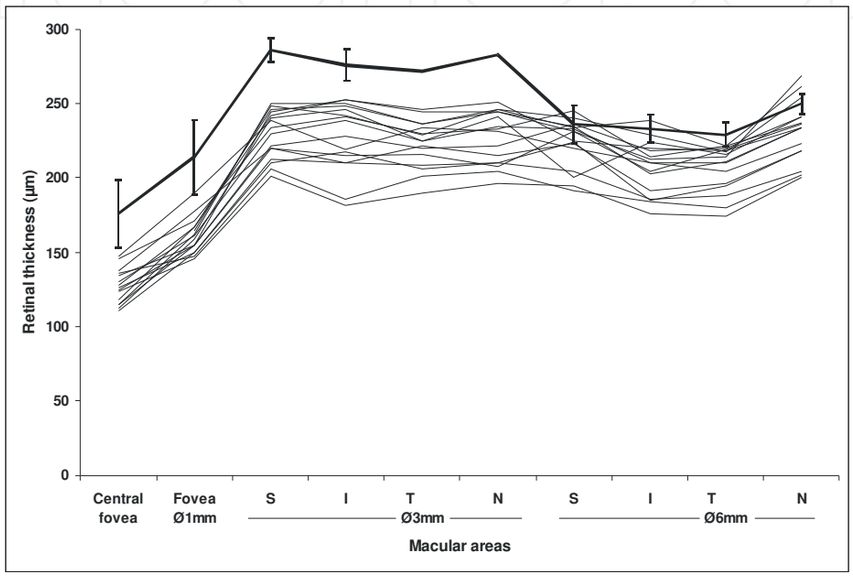

Fig. 8. Optical coherence tomography (OCT) macular thickness measurements of 9 areas in young BD patients (age 9–34 years, n = 8) and controls (in bold), using a Stratus OCT, model 3000 (Carl Zeiss Meditec AG, Jena, Germany): the central foveal, the foveal (Ø 1mm), the inner ring (Ø 3mm), and the outer ring (Ø 6 mm) with Superior area (S), Inferior area (I), Temporal area (T), and Nasal areas (N). The controls are presented with standard deviation shown on the graph. (Figure was published in Arch Ophthalmol. 2010;128(8); 989–995).

The cross-sectional morphology visualized by OCT presents a general thinning of cell layers and a reduced outer nuclear layer (ONL) in the youngest BD patients (9–34 years). Also, a third high-reflectance band, (3rd HRB), found in the younger cases, diminished in younger adults with BD, possibly representing the loss of the outer segment length of photoreceptors, predominately the cones in the fovea measured with OCT (Burstedt et al., 2010; Costa et al., 2004; Sandberg et al., 2005). This finding probably indicates an affection of the cone photoreceptors, and a possible degeneration of the outer segments of cones early in the course of the BD disease. The decreased retinal thickness and degenerative signs in the outer retinal layer were detected early in the course of the disease reported in a compound heterozygous patient with mutations in the *RLBP1* gene (R103W/R234W) (Nakamura et al., 2005). Similar to BD, prominent photoreceptor loss in the foveal and extrafoveal retina even in the youngest patients studied (6–17 years), with relative preservation localized in the

superior–temporal and temporal pericentral retina and the ONL, were demonstrated in the patients with Leber congenital amaurosis, another retinal degenerative disease caused by mutations in *RPE65* gene encoding for a protein active in the visual cycle (Jacobson et al., 2008). How can an early foveal thinning, suggesting early degeneration of cone photoreceptors in BD patients possibly be explained? Studies show that not only the rods but the cones also incorporate 11-*cis* retinoids, derived from the rod and cone visual cycles, in their visual pigments, and when examining the visual cycle in the *RPE-65* and *LRAT* knock-out mice, diseases affecting the visual cycle, a key role for cell survival, was found to be 11-*cis*-retinal bound to cone opsins, important for retinal protein sorting, transport, and targeting (Collery et al., 2008; Zhang et al., 2008).

2.2.3 Morphological findings of retinitis punctata albescens (RPA)

The subretinal white lesions, retinitis punctata albescens, were initially observed in the teens with BD phenotype (Burstedt et al., 1999, 2001, 2010). The RPA spots often dominate in the macula area and adjacent to the arcades, varying from single to multiple generalized white lesions scattered over the posterior pole of the retina. Notably, these changes fade as the progressive retinal degeneration advances (Fig. 9).

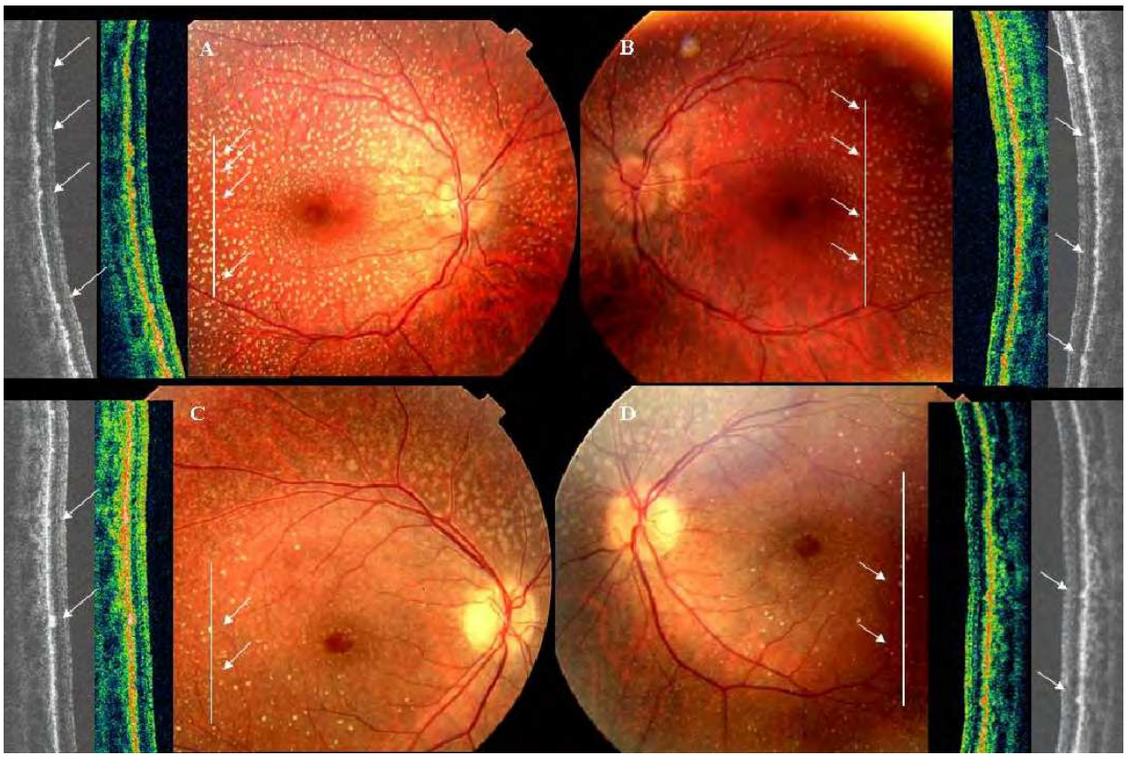

Fig. 9. Colour fundus photo and OCT. Top**:** 23-year-old man; below: 30-year-old woman. The vertical line of the binocular fundus photo with multiple generalized RPA traversing the retina at the temporal area of the macula between the arcades represents the scan lines of the corresponding OCT image presenting cross-sectional, visualized RPA (marked with arrows). (Figure published in Arch Ophthalmol. 2010;128(8);989–995).

RPA or subtle white lesions of the fundus have previously been described in several autosomal recessive RP cases with *RLBP1* mutations, and also in other progressive degenerative diseases, for example, the rhodopsin-related RP (Demirci et al., 2004; Eichers et al., 2002; Fishman et al., 2004; Katsanis et al., 2001; Nakamura et al., 2005; Souied et al., 1996). The localization of RPA lesions and their appearance may resemble more commonly known retinal lesions like drusen in age-related macular degeneration. However, the RPA lesions in BD do not present elevation, disruption, or detachment of RPE. Another finding in AMD is an accumulation of drusen between the retina and choriocapillaris that is shown to interfere with the exchange of nutrients and products close to the drusen, inducing RPE or neural retinal damage with overlaying photoreceptor cell layer thinning, predominately the photoreceptor outer segment affection (Holz et al., 2004; Pauleikhoff et al., 1990; Schumann et al., 2009). Since similar thinning or compression of the ONL overlaying the RPA lesions is observed in BD, a possible cause could be an accumulation of products in the RPE, possibly due to the higher affinity and impaired 11-*cis*-retinal release in the visual cycle in the BD phenotype shown in previous studies (Golovleva et al., 2003; He et al., 2009; Saari, 1990).

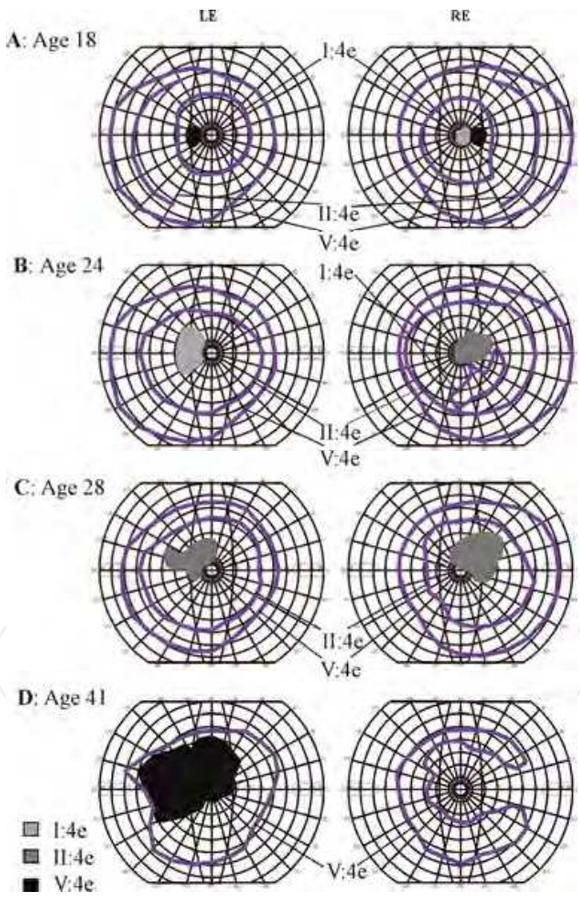

Fig. 10. Progression of visual field defects in a BD case. (A) Small central scotoma is noted in one eye at the age of 18. (B, C) Deeper and larger central-paracentral scotomas develop in young adulthood. (D) In middle age absolute central-paracentral scotomas are present and expanded, finally affecting the peripheral border. (Figure published in Arch Ophthalmol. 2001;119(2):260–267).

2.2.4 Psychophysical findings

Significant foveal depression was found early in BD by testing foveal threshold with Humphrey SITA standard 24-2. The mean deviations in all younger cases show significant loss, indicating an overall depression and/or loss of the central parts of the visual field in BD. Goldmann perimetries are unaffected in the BD cases under the age of 10, and relative parafoveal scotoma and/or ring scotoma is found in the teens, with additional large, deep to absolute central scotomas in both eyes, accompanied by a decrease in visual acuity in adulthood. The visual fields and the development of defects, registered over a time period of 23 years, are presented in Fig. 10.

In the fifth decade, extensive scotomas are present and only peripheral islets of the visual fields remain in middle age.

2.2.5 Angiographic findings

The fundus fluorescein angiograms in the early arteriovenous phase show a diffuse hyperfluorescence in the anatomic macular area, and locally, in the centre of the fovea, also presenting an early retinal thinning. As well as outside the arcades, corresponding to the atrophic areas in the colour fundus photograph, a general hyperfluorescence of granular type appears, indicating a gross atrophy of the pigment epithelium of the entire retina as a common clinical finding in this retinal degeneration (Burstedt et al., 2001).

2.3 Dark adaptometry and electrophysiological findings

2.3.1 Dark adaptometry and electrophysiological findings during standard dark-adapted conditions

Recovery of dark adaptation shows abnormalities of both rod and cone function (Fig. 11).

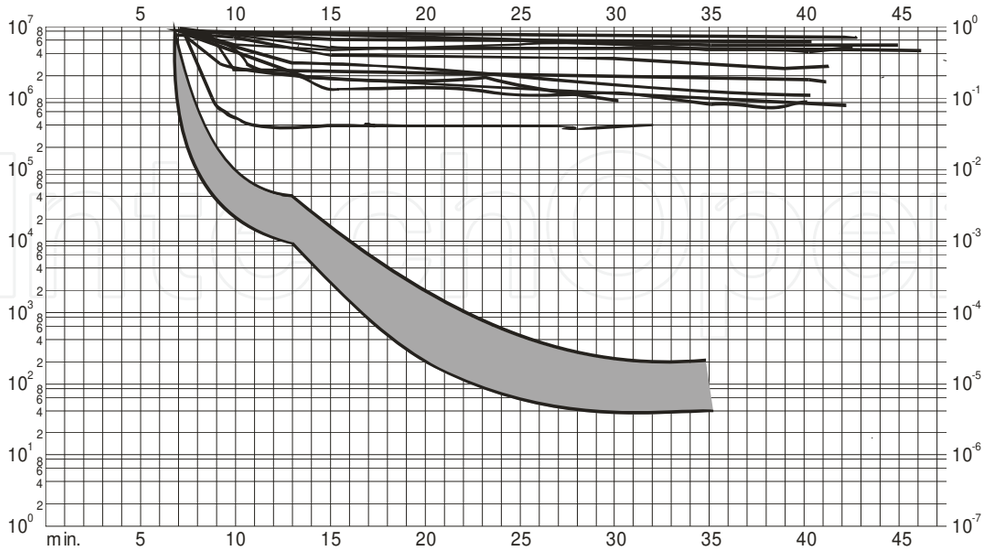

Fig. 11. Results of dark adaptometry in 14 cases aged 8–59 years. The grey area indicates the normal range of recovery of cone and rod sensitivity during dark adaptation for corresponding ages. (Figure published in Arch Ophthalmol. 2001;119(2):260–267).

In the younger patients the rod function is severely affected or absent and the cone adaptation often shows an extremely high, elevated final threshold, with the final dark-adapted sensitivity about four log units higher than a normal range. In elderly affected cases an even more pronounced cone dysfunction is found (Burstedt et al., 2001, 2003).

Full-field, single flash, and flicker electroretinograms (ERGs), including the oscillatory potentials (OPs) are recorded (UTAS-E 2000 LKC Technologies Inc., Gaithersburg, MD) using Burian-Allen bipolar electrodes during standard dark-adapted conditions, according to the recommendations of ISCEV (International Society for Clinical Electrophysiology of Vision). The outcome of the ERGs in all BD cases is subnormal or with non-recordable amplitudes of the rod-isolated b-waves with peak times either within the normal range or prolonged compared to controls (Burstedt et al., 2001, 2003, 2008). Representative full-field ERGs from five individuals from one family are presented in Fig. 12. The mixed rod-cone b-waves amplitudes are subnormal/non-recordable with comparatively short peak times in the younger cases. The amplitudes of the mixed rod-cone a-waves are found to be within the normal range in younger patients and subnormal to non-recordable with a prolonged peak time in adulthood. The amplitudes of the cone b-waves are better preserved and are within the normal range at the very young age, with peak times within the normal range, but prolonged in young adulthood and thereafter. Most of the younger patients had normal amplitudes and implicit times of the 30-Hz flicker ERGs that are within the normal limits, but already in early adulthood subnormal and delayed. The summed amplitudes of the individual oscillatory peaks, representing the Ops, show subnormal values at young age, decreasing with age and becoming non-recordable in BD cases older than 40 years of age.

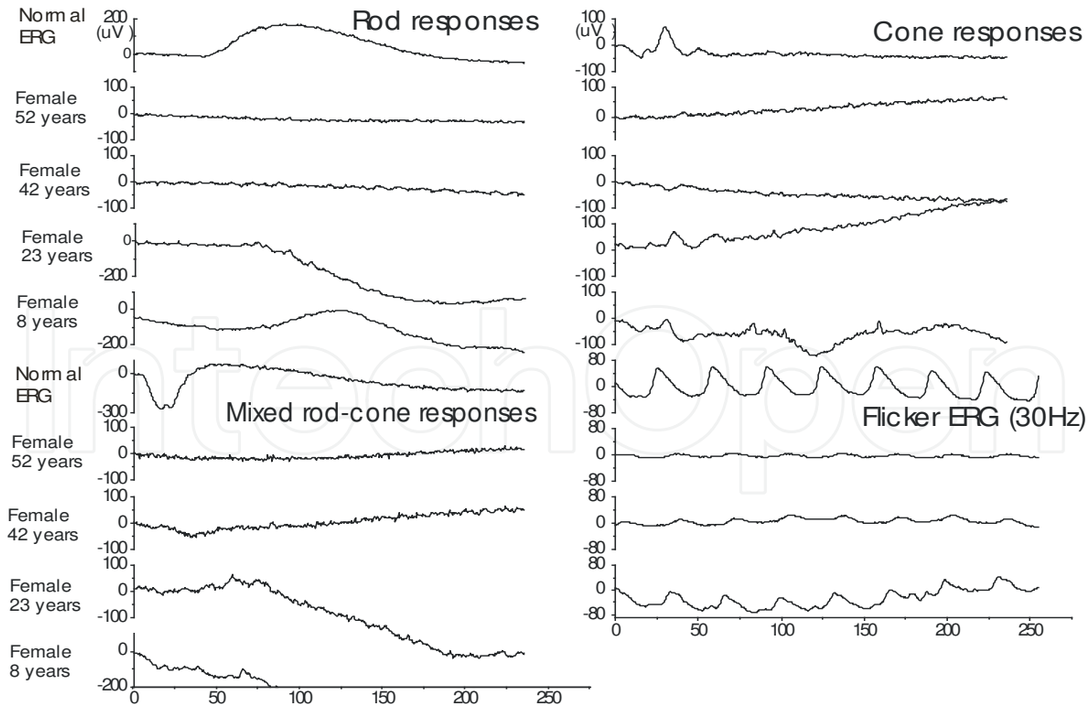

Fig. 12. Full-field ERG responses of the nonaffected individual (above) and BD patients, two elderly, and two younger women (52, 42, 23, and 8 years of age, respectively) from the same family. (Figure published in Arch Ophthalmol. 2001;119(2):260–267).

The ERGs recorded under standard conditions show that the rod and mixed rod-cone b-wave responses are relatively more affected than the mixed a-wave. The cone b-wave and the 30 Hz flicker amplitudes are significantly better preserved than the b-wave amplitudes of the rod and mixed rod-cone responses as well as the a-wave amplitude of the mixed rod-cone response, indicating later disturbance of the cones compared with the rods. At the same time the glial and inner retinal cell types seem to be affected at a relatively early stage in retinal degenerative disease (Burstedt et al., 2003).

2.3.2 Dark adaptometry and electrophysiology findings during 24 h prolonged dark-adaptation conditions

It has been shown that after illumination, the rhodopsin regeneration, 11-*cis*-retinal production, and dark adaptation are delayed by >10-fold in the visual cycle in *RLBP1* knockout mice (Saari et al., 2001). This could also be observed in humans, in six younger adults with BD, examined with a standardized Goldmann-Weekers adaptometer during an extremely prolonged dark adaptation of 24 hours (Burstedt et al., 2003). The extremely slow DA reaches steady state within 5 to 12 hours (Fig. 13); however, the final visual sensory thresholds do not return to normal levels after 24 hours of DA in most of the cases studied, probably affected by the disturbance in the normal function of CRALBP and lack of regeneration of 11-*cis* retinal in the RPE. An apparent plateau of recovery in the dark is observed, and duration for about 1 to 4 hours possibly represents a removal of bleached pigments, which has been suggested to occur in other retinal diseases affecting visual cycle function (Cideciyan et al., 1997). The 11-*cis*-retinal has been estimated to range from about 3.5% to 8% of that in normal subjects (Lamb & Pugh, 2004), with an average level of about 6% in the RPE in the BD phenotype.

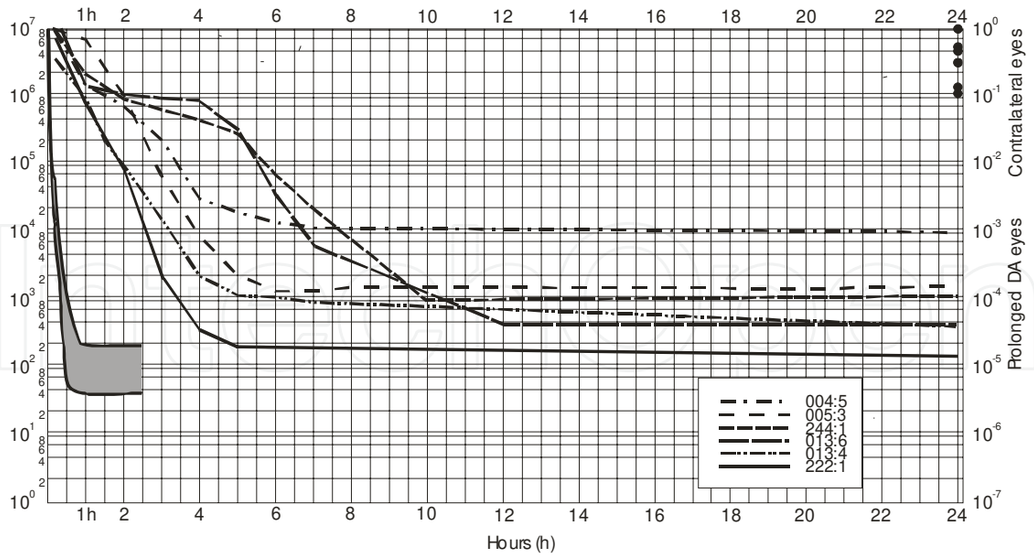

Fig. 13. Results of extremely prolonged (24 h) dark-adaptometry examinations in single eyes in BD patients, n = 6 (age 12–47 years). The final thresholds after 24 h were measured in both the patched single eye and in the unpatched contralateral eye. The values of the unpatched contralateral eye are indicated as filled symbols (•). The shaded area indicates normal range for corresponding age groups. (Figure published in Vision Research 2003:43(24):2559–2571).

Full-field ERGs after 24 hours of extremely prolonged DA were performed to evaluate the capacity of recovery of the whole retinal area and different cell types in BD disease. Six young cases (age 15–30 years) underwent full-field ERGs after 24 hours of DA in one eye and standard DA in the fellow eye. The results could be compared with the effect of the ERG after 10 hours' prolonged DA from a previous study (Burstedt et al., 2003, 2008). Enhanced rod-isolated b-wave after extremely prolonged DA (24 h) shows a sufficient number of rods able to function, generating responses of normal amplitudes even in young BD patients, although still with prolonged peak times (Fig. 14). Since the b-wave of the ERG is a glial response reflecting a depolarization of the Müller cells (Miller & Dowling, 1970), these findings may be correlated to an affected CRALBP function in the Müller cells of the retina or possibly a reversible effect on the second-order ON-bipolar cell function, as a consequence of a change in photoreceptor function.

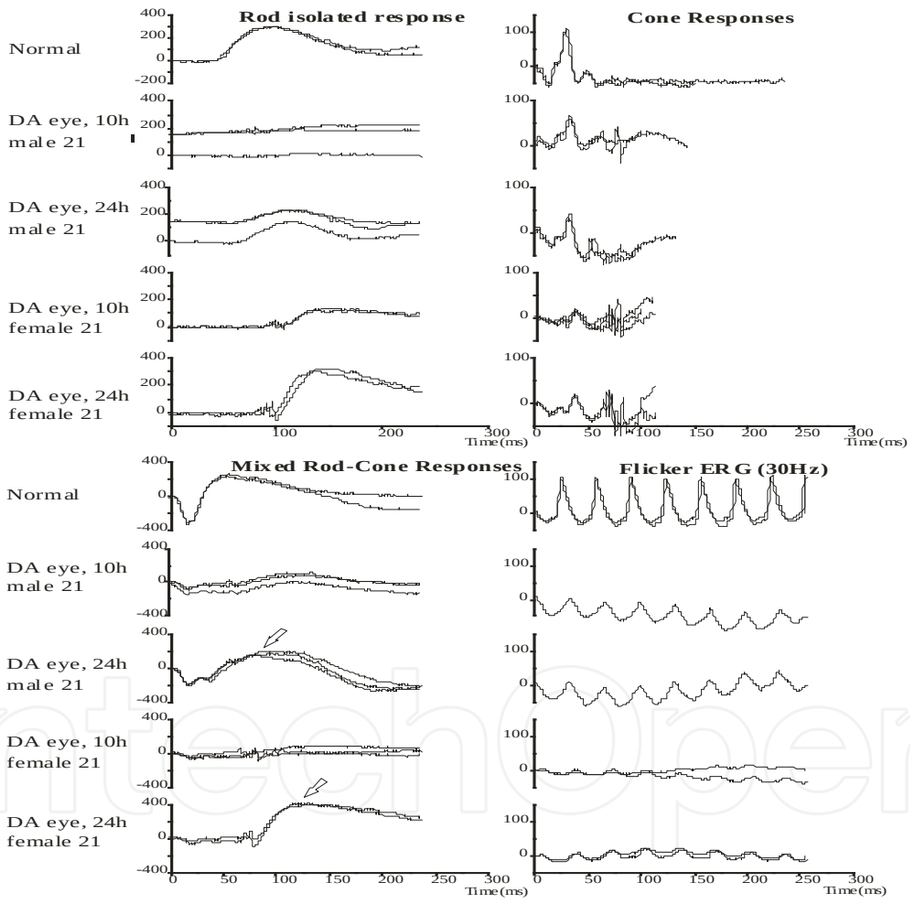

Fig. 14. Full-field electroretinograms of one eye, recorded after prolonged DA (10 h) and after extremely prolonged DA (24 h) in two BD patients (both 21 years old). A 'second', comparatively slowly rising positivity was observed in the mixed rod-cone response (arrows). For comparison, recordings of a normal subject are shown. (Figure published in Doc Ophthalmol. 2008;116(3):193–205).

The mixed rod-cone b-wave amplitude created by depolarization of the Müller and ON-bipolar cells in the inner retina is increased but still subnormal in the majority of patients.

After extended (24 h) DA, the mixed rod-cone a-wave response increases in amplitude to normal values in 4/5 cases with normal or somewhat delayed peak times. As the mixed a-wave shows normalized responses in the majority of the young BD cases examined, and the leading edge of the a-wave mainly represents a light-evoked hyperpolarization of the photoreceptors (Brown, 1968; Jamison et al., 2001; Robson et al., 2003; Tomita, 1965), these data suggest an additional, extremely slow regeneration of photopigments occurring even after 10 h DA. No obvious recovery of the cone b-wave is noted after the extremely prolonged DA (24 h); therefore, a relatively early damage to the cone system in BD patients cannot be excluded. Even though regeneration of opsin photopigments has been demonstrated in cone-dominant retinas, suggesting an interaction between Müller cells and cones in the recycling of visual chromophore (Mata et al., 2002), these electrophysiological results cannot confirm these findings.

In three younger BD patients a late evolvement in the mixed rod-cone response is observed (Fig. 14, arrows). The amplitudes are within the normal range of a mixed rod-cone b-wave, but the peak is extremely delayed (115 ms) compared to normal. This can be related to an enhanced scotopic activity associated with the slow and disturbed regeneration of photopigments. However, the exact origin of this late positive potential observed in BD patients is not known.

In summary, the continuous but slow regeneration of rod photopigments in *RLBP1* mutants presents an additional capacity for recovery of rod function and gain in activity in the inner retinal layers with extremely prolonged dark adaptation, possibly as a consequence of a change in photoreceptor function (Burstedt et al., 2003, 2008).

2.4 Outcome of visual function

Visual loss is an early sign of the BD disease, and individuals of working age may experience important socioeconomic consequences and interference with education as a result of their visual impairment. To provide insight into the perceived visual function of retinitis pigmentosa of Bothnia type, measurements of visual function were associated with the patients' self-assessment of their total self-reported visual function and health-related quality of life, measured with a questionnaire in 49 BD cases (Burstedt et al., 2010). Significant correlation was found between objective visual functions studied, and the subjective visual function. Almost 70% of the variability of the composite score could be explained by WVA and age alone. BD patients' responses to a majority of the questionnaire subscales significantly correlated with several of the clinical vision measures, especially those depending on central vision. Notably, the progressive declines in visual field area did not seem to affect significantly the self-perceived quality of life in patients with this phenotype. This finding might be an indication of ability to adapt to this type of gradual progressive visual field area loss, probably due to use of paracentral preserved areas. The expression of the disease has a significant impact on multiple domains of daily living, but there are no signs of worsening depression related to the increasing visual impairment.

2.5 Tinted contact lenses in BD

Could visual function be improved in BD? With the knowledge of an extremely prolonged dark adaptation (5–12 h) and even further gain of the ERG responses up to more than 12 h in

this phenotype, outcome of wearing of tinted contact lenses during daylight was tested (Jonsson et al., 2007).

Twelve patients with BD were fitted with soft contact lenses tinted dark brown. Outcome of visual parameters, and visual-function questionnaire, were tested before the contact lens fitting and after one month. Visual function was improved by dark-tinted contact lenses, and it was observed that the BD cases with the lowest visual acuity described the most obvious improvement of their visual function and preferred the darker tinted contact lenses; the majority of BD cases from this study have also chosen to continue wearing the brown-tinted contact lenses for several years following the study, possibly indicating a continuous benefit in their daily life over time (authors' comments).

Fig. 15. Contact lenses with clear and different grades of tint were evaluated in retinitis pigmentosa of Bothnia type.

3. Conclusions

The RP population of northern Sweden has given us a unique opportunity to evaluate and compare the phenotypical expression of different *RLBP1* mutations over time. Despite genetic heterogeneity, the clinical expression of different *RLBP1* mutations in northern Sweden presents a unique phenotype of the retinal disease, clearly directing the molecular diagnosis and search for *RLBP1* mutations. It became possible to offer genetic testing among the families related to BD patients and also to the patients with a hereditary form of the recessive form of RP. In BD families we can offer risk assessment to future generations, providing genetic counselling based on molecular testing and clinical findings. There are several approaches to treatment of RP patients, although there are no established standards. Several drugs and nutritional supplements such as vitamin A palmitate, ascorbic acid, docosahexaenoic acid and others were evaluated with contradictory outcomes in different

studies. Vitamin A palmitate, the most-used nutritional supplement, is shown to slow the rate of retinal degeneration, but this type therapy is not without controversy (Shintani et al., 2009). Gene therapy, which replaces or turns off the mutant disease-causing gene, represents another option for treatment. Several years of basic research of *RPE65*-Lebers congenital amaurosis, a retinal disease affecting the visual cycle, by several independent research groups, resulted in clinical trials of human gene therapies during recent years, demonstrating short-term evidence of visual gain (Jacobson & Cideciyan, 2010; Musarella & MacDonald, 2011). Therefore, we suggest that for treatment of BD patients, gene therapy is the most promising option among other concepts in the treatment of retinitis pigmentosa.

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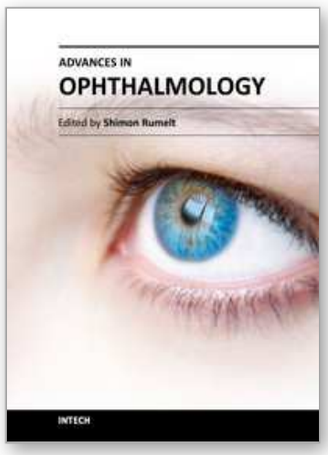

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