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BOTHNIA DYSTROPHY

A CLINICAL, GENETICAL AND ELECTROPHYSIOLOGICAL STUDY

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To Magnus and my family

ABSTRACT

A high frequency of retinitis pigmentosa (RP) is found in Northern Sweden. In an inventory of autosomal recessive RP patients in Västerbotten County, a great number of cases with a unique phenotype was noticed, denoted Bothnia Dystrophy (BD). The aim of the study was to describe the phenotype, to determine the chromosomal location, and to identify the gene.

Patients typically show night blindness from early childhood. Symptoms of defect macular function with a decrease of visual acuity can appear in early adulthood. The retinal fundus shows irregular white spots in a central, and parafoveal pattern along the arcades. Centrally areolar maculopathy develops and round circular atrophies are observed in the periphery.

The disease was shown to be associated with a missense mutation in the *RLBP1* gene resulting in an amino acid substitution (R234W) in the cellular retinaldehyde-binding protein (CRALBP). The R234W mutation was found in a homozygous state in 61 patients affected with BD. Ten patients were heterozygous for the R234W mutation, and presented a similar phenotype. No additional mutations in the coding sequence or exon-intron junctions were found. CRALBP is localised in retinal pigment epithelium (RPE), and Müller cells of the retina. In the RPE, CRALBP functions as a carrier protein for endogenous retinoids.

Dark adaptometry and electrophysiologic testing showed an initial loss of rod function followed by a progressive reduction of the cone responses in older ages. A compromised rod function, dysfunction of the Müller cells, and indications of a disturbed function of the inner retina were found. With prolonged dark adaptation, a gradual increase in retinal sensitivity to light and an improvement of the ERG components occurred. The findings indicate a prolonged synthesis of photopigments, retardation of the visual process in the retinal pigment epithelium and a loss of retinal cells probably starting at a relative early age in BD.

To evaluate the subjective visual function in BD patients, a battery of objective tests of visual function and composite score of the 25-item NEI-Visual Function Questionnaire (VFQ-25) were analyzed. We found that weighted distance logMAR visual acuity (WVA), had the strongest association with subjective visual function, and that there was a considerable loss of subjective and objective visual function with increasing age in BD patients. The prevalence of BD is as high as 1:3600 in Västerbotten County.

The possibility that recycling of retinoids localized in the RPE might be impaired in BD might give future therapeutic possibilities. Due to the large and clinically well-characterized set of patients with this disease, they constitute a suitable study group.

CONTENTS

ABBREVIATIONS

AD-autosomal dominant inherited

AR-autosomal recessive inherited

BD-Bothnia Dystrophy

CRALBP-cellular retinaldehyde-binding protein

CRBP-cellular retinal-binding protein

CS-contrast sensitivity

DA-dark adaptation

EOG-Electrooculogram

ERG-Electroretinogram

IRBP-Intraretinal-binding protein

ISCEV-International Society for Clinical Electrophysiology of Vision

logMAR -logarithm of minimum angle of resolution

LRAT-lecithin:retinol acyl transferase

NEI-VFQ-25-National Eye Institute-Visual Function Questionnaire

NFRCD-NewFoundland rod-cone disease

OAT-ornithine aminotransferase

OP-oscillatory response

Ops-oscillatory potentials

PCR-polymerase chain reaction

RBP-serum retinol-binding protein

RFLP-restriction fragment length polymorphism

RP-Retinitis Pigmentosa

RPA-Retinitis punctata albescens

RPE-retinal pigment epithelium

SSCP-single-stranded conformational polymorphism analysis

SPSS-statistical package for the social sciences for MS Windows

VA-visual acuity

VF-binocular visual field

VRQL-visual related quality of life

WCS-weighted logMAR low contrast visual acuity

WVA-weighted distance logMAR visual acuity

ORIGINAL PAPERS

I. Burstedt MSI, 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(5): 995-1000

II. Burstedt MSI, Forsman-Semb K, Golovleva I, Janunger T, Wachtmeister L, Sandgren O. Ocular phenotype of bothnia dystrophy, an autosomal recessive retinitis pigmentosa associated with an R234W mutation in the *RLBP1* gene. Arch Ophthalmol. 2001; 119(2): 260-7

III. Burstedt MSI, Sandgren O, Golovleva I, Wachtmeister L. Retinal function in Bothnia Dystrophy. An electrophysiological study. Vision Research 2003; 43(24): 2559-2571

IV. Burstedt MSI, Kadzhaev K, Beckman L, Sandgren O, Golovleva I. Clinical and genetic studies on heterozygous individuals with Bothnia Dystrophy. Manuscript.

V. Burstedt MSI, Mönestam E, Sandgren O. Association between specific measures of vision and Vision-Related Quality of Life in patients with Bothnia Dystrophy, a defined type of Retinitis Pigmentosa. Submitted.

INTRODUCTION

GENERAL BACKGROUND

The retinal degenerative diseases include a group of genetic dystrophies leading to progressive photoreceptor degeneration and, thus visual impairment. The dystrophies differ from each other in the mode of inheritance, the fundus appearance, the visual disability experienced by the patients, and the objectively measurements of vision. For a correct diagnosis, the right questions and examinations are essential. To estimate the future visual handicap, the ophthalmologist needs to know the mode of inheritance, the clinical phenotype, and the rate of progression of the patient's symptoms. Adequate information to the patient is essential, and the fate of future generations is an important and difficult question to be answered. In order to give the answers to all these questions a well-defined classification and a phenotypical description of the retinal disease is needed. The genetical classification of patients is essential not only for appropriate counseling but also for identification of patients and future research in the disease.

The developments in molecular biology have had a high impact on research in the field of retinal dystrophies in the last decade. By using different molecular biological strategies such as genetic mapping, and mutation analysis, knowledge has been obtained about several inherited retinal diseases. Great efforts have been made in the biochemical research to find the proper function of the affected protein in the visual cycle and to lead us to further knowledge and understanding in all the fields of research associated with the retinal degenerations.

A high frequency of retinal dystrophies in Northern Sweden and the observation of a unique phenotype, Bothnia Dystrophy have given us an exclusive possibility to combine clinical observations with modern molecular technique in this study.

The normal retina

The retina has three nuclear (cellular) layers, interspersed with two synaptic (plexiform) layers. After passage through the optics of the eye, light enters the normally transparent retina at the ganglion cell side, penetrates through the entire retina and is captured by the most distal retinal elements (the photoreceptor outer segments). All photoreceptors have their nuclei in the outer layer. The inner nuclear layer contains three other types of neuronal cell bodies: the horizontal, bipolar and amacrine cells. There are in addition, prominent glial elements (Müller cells) that extend vertically through the entire retina. The inner retina is nourished by retinal blood vessels, whereas the outer retina, including the photoreceptors, depends on choroidal blood flow for its nourishment (Dowling et al, 1984).

The retinal pigment epithelium (RPE) is derived from the same neural tube tissue as the sensory retina. The cells are packed with the black pigment melanin, from which it gets its name. A RPE cell has an apical portion with numerous long microvilli facing the photoreceptors, and basal portion facing Bruchs membrane. Since the RPE stands between the choroidal vessels and the photoreceptors, it is evident that this layer of cells plays an important role in photoreceptor nourishment, including retinoid delivery (Saari, 1994).

The photoreceptor cells initiate the visual process by converting the image into discrete neural signals. The distribution of rod and cone photoreceptors across the retina is highly asymmetric. Red and green sensitive cones are concentrated in the fovea (Curcio et al, 1987). The high acuity vision is achieved by "direct wiring" of red- and green-sensitive cones present with highest density in the foveal region. Blue sensitive cones are found throughout the retina. Rod photoreceptors are distributed outside the foveolar region and are "gang

wired" with the output of as many as 1000 rods feding into a single ganglion cell. This neural network increases visual sensitivity to light with a corresponding loss of acuity (Saari, 1994).

Müller cells are the principal glial cells of the retina. They are oriented radially within the neural retina, extending from the photoreceptor inner segments to the vitreal border of the retina. Müller cells are thought to regulate extracellular ion levels, active uptake of neurotransmitters, and to give structural, metabolic support of neurons and neuronal guidance during development (Albert et al, 1994).

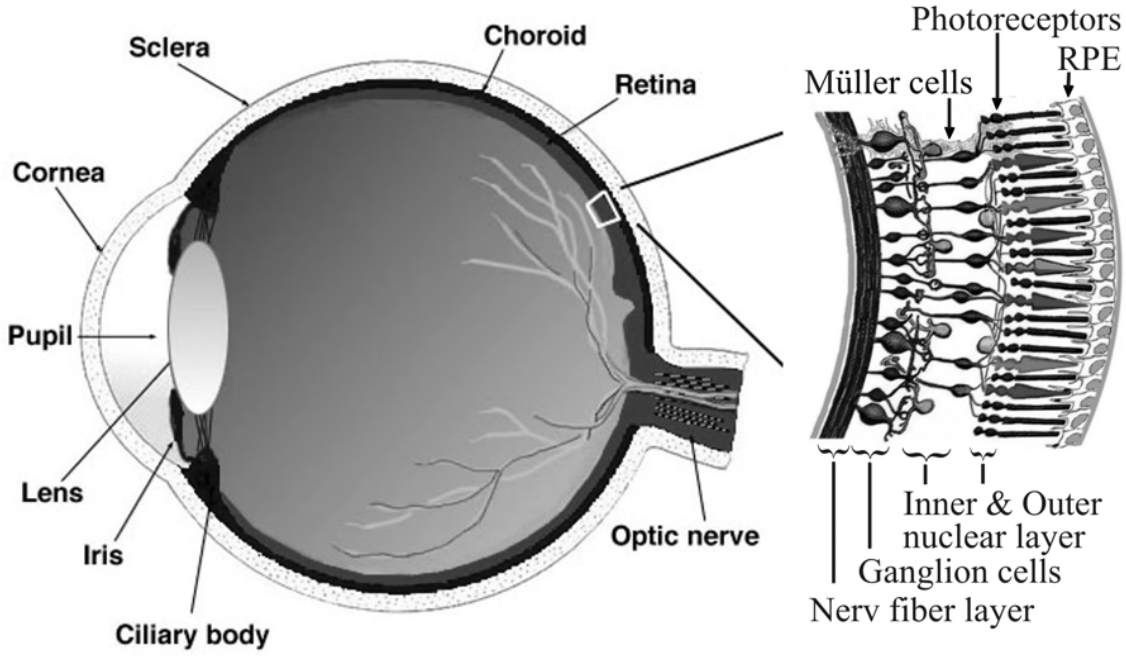

Figure 1: The eye, and the nuclear layers of the retina. Modified from http://www.visres-interactivemeeting.com/cycle.htm, used by permission.

The visual process

The chromophore derived from vitamin A is complexed with a protein component (an opsin) and named rhodopsin (visual purple). The absorption of a photon by rhodopsin triggers a process of signal transduction, *the phototransduction*. The process is a cascade in which photoactivated rhodopsin sets in motion a chain of sequential activations of other components of the rod photoreceptor outer segment (ROS), leading ultimately to a decrease in the conductance of the plasma membrane of the cell to cations. Intermediates in the cascade are metarhodopsin II, the G-protein transducin, cyclic-guanosine monophosphate phosphodieesterase (cyclic-GMP) and a cyclic-GMP-gated cation channel. Amplification results in a considerable gain in the system (Saari, 1994).

Traditionally *the photoisomerization* has been referred to as "bleaching" since illumination of the retina cause a progressive color change from purple (11-*cis*-retinaldehyde opsin), to yellow (all-*trans*-retinaldehyde opsin) and to colorless (all-*trans* retinol).

The visual cycle describes the reactions utilised to produce the critical 11-*cis* retinoid needed from all-*trans*-precursors. The process is enzymatic and located in the retinal pigment epithelium (RPE). It is the site of isomerization and oxidation of the all-*trans* retinol to 11-*cis* retinal as well as the estherification, and storage of retinoids for use in the visual cycle. All-*trans* retinol enters the RPE from movement both across the apical membrane and the basal

membrane, where all-*trans* retinol circulates in the blood bound to serum retinol-binding protein (RBP). A receptor-mediated mechanism of uptake in the RPE is driven by a protein (RPE65). In addition it is probable that some retinoid enters the RPE through the phagocytosis of shed tips of the outer segments (Crouch et al, 1996).

All-*trans*-retinol enters the RPE where it is esterified by the enzyme LRAT (lecithin:retinol acyltransferase) and then the all-*trans* retinyl ester is converted into 11-*cis* retinal by isomerisation, supported by RPE65, and hydrolysis. Retinol esterification is considered a "detoxifying reaction" as it forms a relatively non-toxic product that can be stored compared to toxic free retinoids. The oxidation of 11-*cis* retinol to 11-*cis* retinal utilizes an 11-*cis* retinol dehydrogenase (11cisRDH). The 11-*cis* retinal then diffuses into the photoreceptor cell where it associates with opsin to regenerate the visual pigment (Saari, 2000).

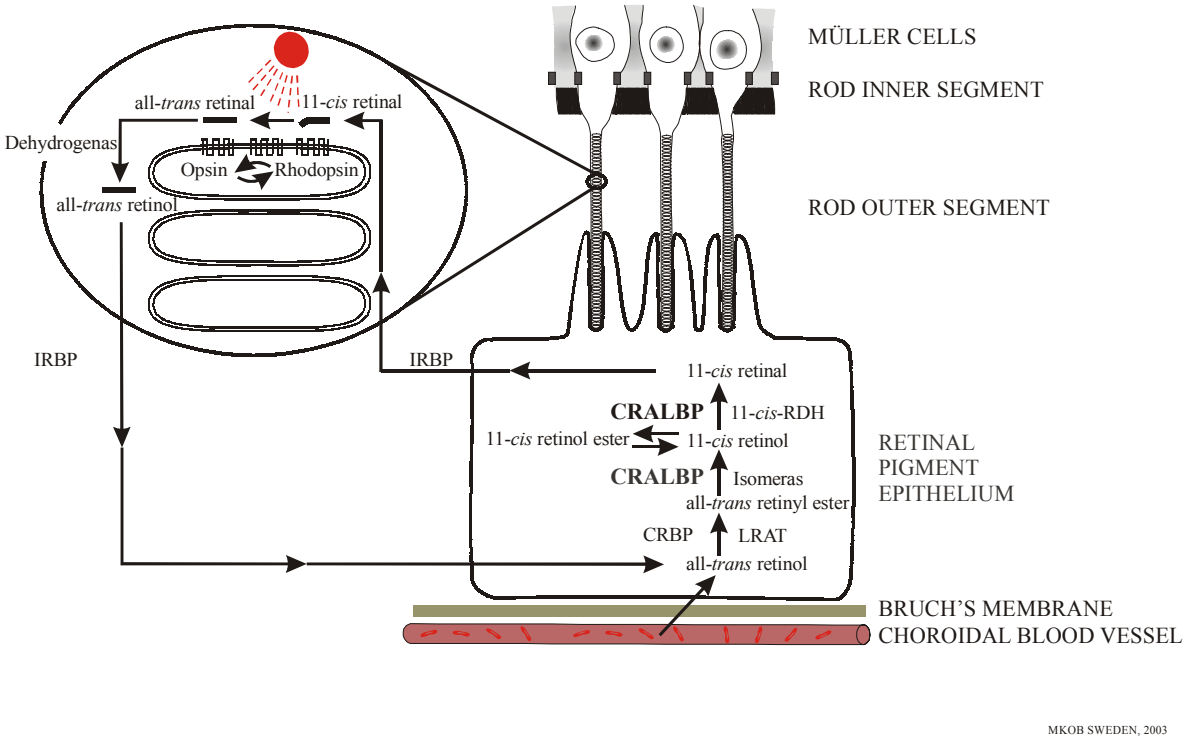

Figure 2: Overview of the phototransduction, the photoisomerization and the visual cycle.

In the RPE, cellular retinaldehyde-binding protein (CRALBP) is known to play a crucial role acting as a carrier protein in the visual cycle and the regeneration of rod visual pigment (Bok, 1990;Saari et al, 1994;Rando, 1996;Crouch et al, 1996). CRALBP has also been purified from Müller cells (Saari et al, 1982;Bunt-Milan et al, 1983). In the Müller cells there seems to be some retinoid metabolism and the presence of 11-cis retinoids strongly suggests that it is related to the visual cycle (Saari, 2000). CRALBP has also been localized in the ciliary body pigment epithelium, the outer epithelium of the iris, the cornea, the optic nerve, and the pineal gland. (Futterman et al, 1977; Bunt-Milan et al, 1983; Eisenfeldt et al, 1985; Bridges et al, 1987;Sarthy, 1996;Saari et al, 1997). Retinoid interactions determine the function of the CRALBP in the rod visual cycle where it affects the enzyme activity in four enzymes of the visual cycle in vitro (Saari et al, 1994; Winston et al, 1998; Stecher et al, 1999)(Figure 2). In vivo studies has shown that isomerization of all-*trans*- to 11-*cis*-retinol is substantially impaired in the absence of CRALBP (Saari et al, 2001).

Six CRALBP mutations have been linked to retinal pathology, including three missense mutations (R150Q, M225K and R234W), a frameshift mutation and two predicted splice junction alterations (Maw et al, 1997; Burstedt et al, 1999; Morimura et al, 1999; Eichers et al, 2002). The nightblindness reported in human patients with CRALBP mutations could result from defects in any of the enzymatic activities (Saari et al, 2001).

PSYCHOPHYSICAL AND ELECTROPHYSICAL TESTS OF THE RETINA

Dark-adaptometry

Dark adaptation is measured by dark-adaptometry (see methods). The increase in sensitivity, which occurs during dark adaptation, is usually expressed in terms of light intensity just visible (the threshold) at any given moment. The threshold is plotted, and the well-known dark adaptation curve is obtained. This curve is made up of two parts, the first part being generally considered to represent cone adaptation and the second rod adaptation (Karpe et al, 1948). The cone branch is obtainable from the rod-free fovea. The rod branch is obtainable from all parts of the retina where rods are present. This branch exhibits the spectral sensitivity of twilight vision, which corresponds to the absorption spectrum of rhodopsin. The time course of rhodopsin regeneration and the return of the sensitivity of the rod system are generally correlated (Rushton, 1961). However, the recovery of the rod sensitivity threshold after very intense bleach is also partly dependent on removal or inactivation of bleaching intermediates or photoproducts within the photoreceptors (Pepperberg, 1984).

Electroretinogram (ERG)

Electroretinography is the recording of the action potentials elicited within the retina upon its stimulation by light. A basic protocol, the ISCEV standard, has been agreed upon and later updated in 1999, to be able to compare recordings throughout the world, (Marmor et al, 1999). The electroretinogram (ERG) is the sum of different electrical responses occurring in various structures, and layers of the retina. Granit was the first one to analyze its major components (Granit et al, 1937). Depending on light stimulus and adaptation conditions chosen, there is a variation in waveform of the ERG. The most important components recorded in clinical practice are the a-, and b-waves. Their amplitudes (μV) and implicit times ((ms) time from stimulus to maximum response) are measured. Special stimulus- and background-light intensities are chosen to separate the responses of the rods from those of the cones. Five different responses are routinely recorded from the patient according to the ISCEV standard (Figure 3).

The b-wave of the ERG is a glial response that reflects a depolarization of the Müller cell as a result of a light-evoked rapid increase of potassium concentration in the inner retina. The b-wave also indirectly represents the activity of the ON-bipolar cells (Miller et al, 1970;Dick et al, 1978;Xu et al, 1994a-b). The dark-adapted mixed rod and cone response conveys information about the total retinal function. To evaluate the function of the outer retina, the a-wave is measured. The slope of the a-wave mainly represents a light-evoked hyperpolarisation of the photoreceptors (Tomita, 1965;Brown, 1968;Heynen et al, 1985). The oscillatory response (OP), reflecting the neuronal activity at the level of the inner plexiform layer (IPL), is probably initiated by the amacrine cells (Ogden et al, 1971; Ogden, 1973; Wachtmeister, 1998, 2001). The light adapted single flash response is recorded to demonstrate the function of the cones. Finally stimulation with a 30Hz flickering light in a light adapted state isolates the cone response; its amplitude and peak time representing the function of the cones in the retina.

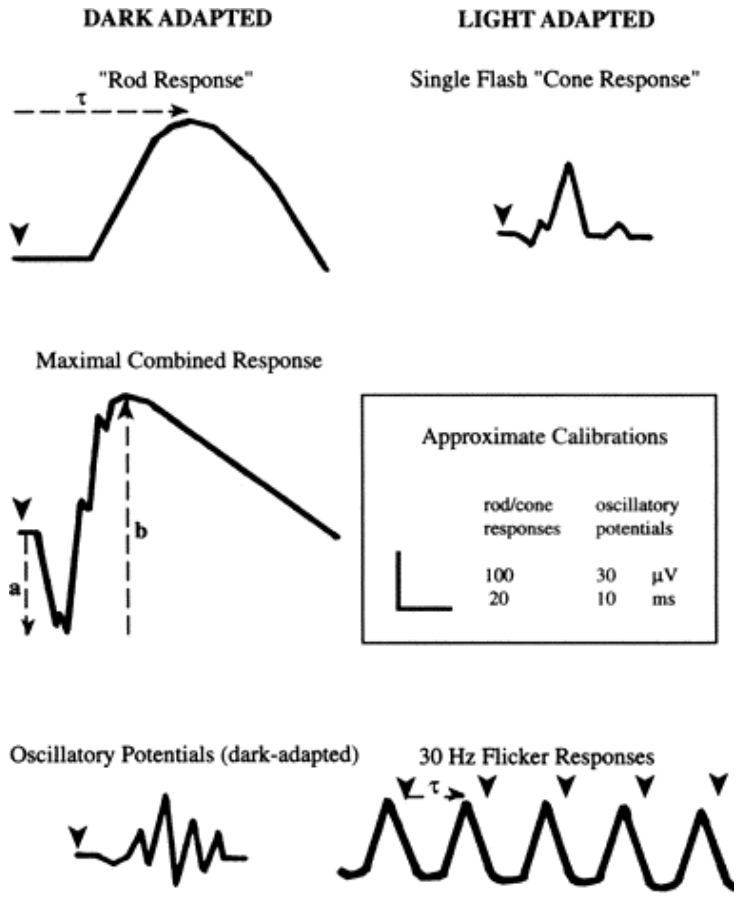

Figure 3: Diagram of the five basic ERG recordings defined by ISCEV standard.(From Marmor et al 1999. used by permission)

Electrooculogram (EOG)

Electrooculography measures the standing potential, which is present between the anterior and the posterior pole of the eye and is generated by the RPE. The nature and clinical utility of the electrooculogram (EOG), was described by Arden in 1962 (Arden et al, 1962). After pre-adaptation to a bright light, the patient alternately looks between two points 20 ^{0} apart; first in darkness and then in light illumination. As the patient moves the eyes horisontally, a square wave signal is generated. The amplitude of this signal, changes with light adaptation normally being twice as large in the light adapted state as in the dark, is named the Arden ratio.

RETINAL DYSTROPHIES

The retinal dystrophies are a complex group of hereditary diseases. Different structures of the retina can be affected like the photoreceptors, the RPE or the choroid as well as retinal processes, like the phototransduction or the retinoid cycle. This can cause various defects in retinal function resulting in low VA, defect colour sense, defect night vision and impairment of the visual fields. The classification of hereditary degenerative retinal dystrophies has earlier been based on clinical and electrophysiological findings. Progress in molecular biology has shown that identical dystrophies clinically can result from mutations in different genes; genotypes, and mutation in the same gene resulting in different expression of a retinal disease; phenotypes. For overview of, some of the diseases affecting the retinoid part of the visual cycle and their animal models see Figure 4. An updated list of disease loci is available on the RetNet web site; http://utsph.sph.uth.tmc.edu/retnet

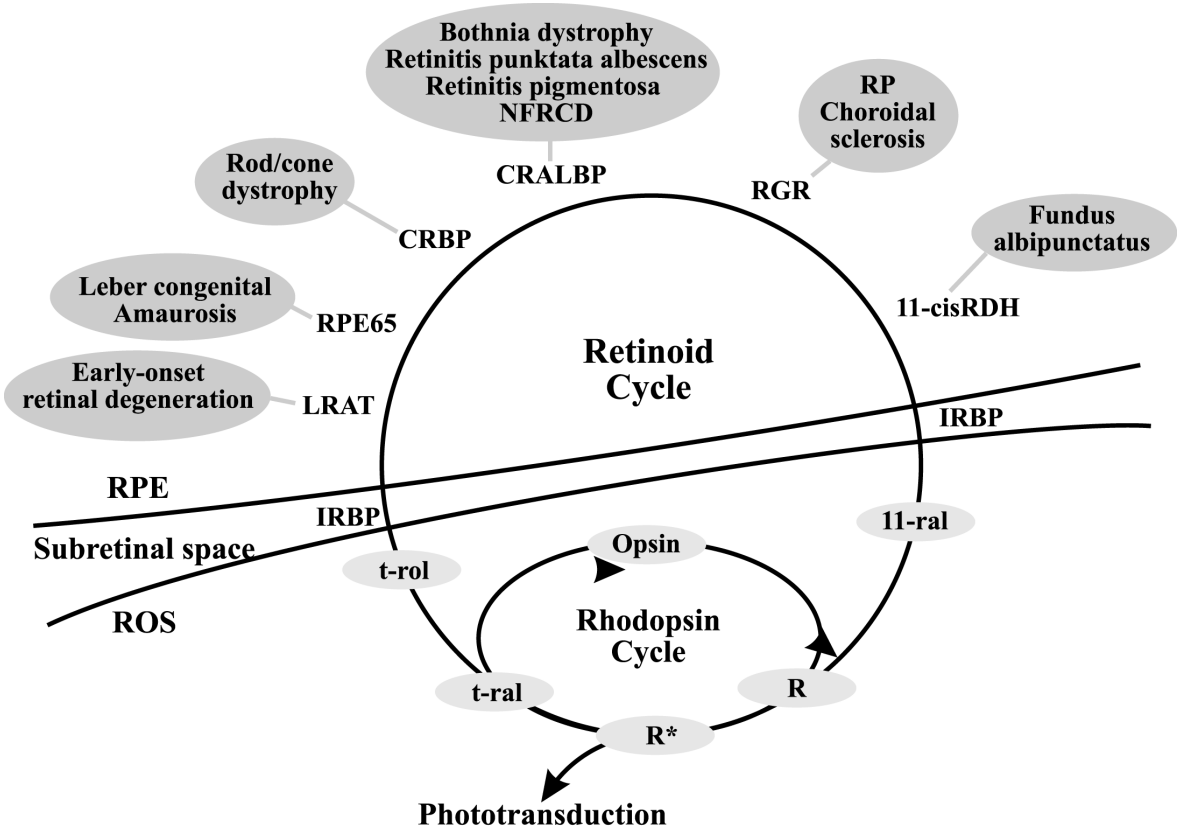

Figure 4:. Summary of retinal proteins affected in the visual cycle and the known associated retinal diseases. ROS, rod outer segment where R*, activated rhodopsin release all-*trans* retinal (t-ral) reduced to all-*trans* retinol (t-rol) for further transfer with interphotoreceptor retinoid-binding (IRBP) to RPE(retinal pigment epithelium) through subretinal space. The abbreviations used are: LRAT, lecitin:retinol acyltransferase; RPE65, RPE specific protein; CRBP, cellular retinoid-binding protein; CRALBP, cellular retinaldehyde-binding protein; RGR, RPE-retinal G-protein coupled receptor and 11-cisRDH, 11-*cis* retinol dehydrogenase. Modified from http://www.visres-interactivemeeting.com/cycle.htm, used by permission.

Retinitis pigmentosa (RP)

There is a great variability among RP patients, but the typical RP patient complains of night blindness, noticeable symptoms of visual field loss in adulthood, and finally severe visual disability with tubular fields, or no functional vision in the late adulthood. Slightly less than half of RP patients will develop cataracts, usually in advanced stages of the disease (Heckenlively, 1982). RP is a heterogeneous group of disorders, with multiple genetic forms and can show autosomal dominant (AD), autosomal recessive (AR) and x-linked recessive inheritance.

Studies from Maine (USA) have shown a prevalence of patients affected with non-syndromic RP of 1:5193 and a prevalence of 1:4756, including Usher and Bardet-Biedl syndromes. (Bunker et al, 1984). Studies from Birmingham (UK), showed a prevalence of total RP patients of 1:4869 (Bundey et al, 1984). The prevalence of RP in Denmark is reported to be 1:3943, including Usher, congenital amaurosis of Lebers, choriodermia, gyrate athrophy and Bardet-Biedl) (Haim et al, 1992). About 20% of patients showed AD inheritance and about 10% x-linked inheritance and the rest were considered AR or simplex cases in these reports.

Early in the disease the retinal degenerations, like RP, may show no or mild pigmentary changes in the fundus. As the disease progresses more pigmentary disturbances in the form of bone corpuscular-like clumps and strands of black pigment occur, most prominently in the periphery and often in a perivascular pattern due to pigment within the vessel walls. In many cases those areas of the fundus not involved with pigmentary deposition show a moth-eaten appearance or a salt-and-pepper scattering of pigments. In advanced cases the optic disc often appears with a "waxy pallor" in RP (Heckenlively, 1988).

Poor night vision is often the first subjective symptom in retinal degenerations primarily affecting the rods. Cone and cone-rod dystrophies do not initially affect night vision until late stages. Cone adaptation and the early part of the recovery of rod sensitivity follows the normal time course, but the later phase of rod adaptation is often markedly elevated or prolonged in RP (Steinmetz et al, 1992). Among the many abnormalities of dark adaptation described in retinal diseases, one of the classic patterns in an increase in the time to rod-cone break (ie prolongation of the cone plateau), and a slower final recovery which suggests a disturbance in the rod system (Cideciyan et al, 1997).

In RP preserved VA can occur until late in the disease when last of the cones in the macula degenerate (Berson et al, 1985). No significant difference was found regarding VA in RP patients in relation to the duration of night vision loss in the three mendelian types. (Pearlman, 1979). Other studies show that central VA loss was mildest in ADRP, and most severe in x-linked recessive inheritance (Fishman et al, 1979). Marmor 1980, presented data where AR patients clearly had less preserved VA with increased age, compared with ADRP patients (Marmor, 1980).

Progressive visual field loss is one of the cardinal features of RP, and is a necessary finding in order to make the diagnosis. Patients with hereditary retinal diseases normally have symmetrical findings between the eyes. In RP the early findings typically include slight loss in the superior peripheral field and scotomatous areas in the mid-equatorial field. With time, multiple scotomatous areas become more confluent, so that partial to full-ring scotomata emerge. As the disease advances, the superior and nasal fields are lost, leaving a central island of field with elongated temporal islands. Other retinal dystrophies like cone, cone-rod dystrophies, choroidal or RPE affecting diseases usually cause central visual field loss.

The most common color vision abnormality seen in RP is a tritanomalous change (blue-yellow axis). When an atrophic macular lesion was present, or the VA was less than 20/30, no patient had normal color vision in RP patients. (Fishman et al, 1981). Patients with cone- and cone-rod dystrophies usually show protano-deuteranopsi as color vision defect Krill1973 (Krill et al, 1973).

Functional assessment and quality-of life surveys are gaining in acceptance as effective evidence-based methods of measuring patients' well-being and limitations. Only a few studies regarding Vision-Related Quality of life (VRQL) and patients with retinal disorders like RP have been published (Szlyk et al, 1997;Turano et al, 1999). The visual acuity (VA) was most strongly related to the patients`ratings of their difficulty in performance in patients affected with typical RP, and Usher syndrome type 2. Visual field area was also associated with patients` self-assessments but not as strongly as VA. In this report it was also shown that overall ERG amplitudes showed the smallest relationship with patients` self-report (Szlyk et al, 1997). In the second study on RP patients log MAR visual acuity, log contrast sensitivity, and log retinal area together accounted for 57% of the variability of the RP-patients self-assessed visual function (Turano et al, 1999).

In 1945, Karpe reported that the ERG was absent in patients with RP, and from that point the ERG evolved to become an important test in the diagnosis (Karpe, 1945). However not all RP

patients had non-recordable ERGs, and it was shown that the responses was proportional to the severity of the disease (Björk et al, 1951). In looking for the rod-cone and cone-rod patterns, the cone and rod b-wave amplitudes are evaluated. In most RP patients the rod-mediated ERG is more severely affected than the cone mediated ie. rod-cone degenerative disease. The implicit time has also been investigated. In the panretinal degenerations, progressive amplitude reduction often leads to delays in the implicit times, specifically, photopic implicit times. The reduced and delayed 30 Hz flicker ERGs can be detected years to decades before other symptoms emerge (Berson, 1993).

The EOGs in RP patients with rod-cone affection show Arden ratios of 100 to 120% while patients with cone-rod affection commonly have higher values ranging from 130 to 150%. Advanced RP patients of both types present values near 100% (Heckenlively, 1988).

Differential diagnosis of RP

In spite of progress in molecular genetics, the clinician still has to relay on clinical and electrophysiological examinations in the diagnosis of retinal dystrophies, as the genetic analyses performed in most cases are done within research projects. A few retinal dystrophies with similar phenotypes to BD, and of interest regarding retinal fundus findings, psychophysical, and electrophysical parameters are listed below.

Gyrate atrophy

Gyrate atrophy is an AR inherited dystrophy with an ornithine aminotransferase (OAT) enzyme defect, giving high levels of ornithine in the plasma and urine. Gyrate atrophy presents distinctly defined round shaped retinal atrophies in fundus similar to BD. These circular areas of total vascular atrophy of the choroid and retina in the mid and far periphery are described already in the first decade of life. The ERG is severely abnormal and the rod responses appear more affected compared to those from the cone system. (Weleber, 1991).

Choroidermia

Choroidermia is a x-linked dystrophy with mutations in the CHM gene. It is characterized by diffuse progressive degeneration of the RPE and choriocapillaris and is first manifested as mottled areas of pigmentation in the macula and equatorial region. Patients with choroidermia tend to have a generalized depigmentation of the RPE. In spite of considerable differences between choriodermia and RP the diagnosis could be set wrong and the ERG finding can be similar with a rod function in excess of cone function, but both photopic and scotopic ERGs are abnormal. Fluoroscein angiogram described early in choriodermia can show diffuse hyperfluorescence as in BD patients. The finding most likely to confirm the diagnosis of choriodermia is the mode of inheritance affecting male predominantly with slow deterioration of visual function and discrete changes in the female carriers (Kärnä, 1986).

Sorsby´s fundus dystrophy

Sorsby´s fundus dystrophy is AD inherited and caused by mutations in the *TIMP3* gene. Patients initially experience night blindness, then loss of central vision after the fourth decade of life, either from subretinal neovascularization or geographic atrophy of the outer retina and inner choroid. Prolonged dark adaptation and reduced rate of rhodopsin regeneration is described in this disease (Steinmetz et al, 1992).

Fundus albipunctatus

Fundus albipunctatus is defined as a variant of congenital stationary nightblindness with an autosomal recessive inheritance. The fundi of these patients have a characteristic appearance with large number of discrete, yellow-white lesions at the level of RPE. An unusually long dark-adaptation period is needed to obtain the maximum normal scotopic response. The clinical course has been considered to be stationary with normal visual aquity, visual field and color perception. The 11-*cis*-retinol dehydrogenase gene (RDH5) is identified as the mutated gene in these patients. Recent studies have shown fundus albipunctatus with or without macular dystrophy in this phenotype (Nakamura et al, 2003).

Vitamin A deficiency and abetalipoproteinemia

The first measurable clinical manifestation of these aquired diseases is nightblindness with abnormal dark adaptation. In severe cases fundus changes similar to albipunctate dystrophy may appear. Systemic manifestations of vitamin A deficiency and other ocular findings such as xerophthalmia and keratomalacia distinguishes it from RP. Abetalipoproteinemia may include ERG abnormalities, peripheral visual field loss, pigment deposition, and bone spicule formation. Many patients have a reversal of symptoms but not of pigment deposition, on vitamin A therapy. (Gouras et al, 1971).

AIMS OF THE STUDY

To determine the chromosomal location and to identify the gene causing Bothnia dystrophy (BD).

To describe the clinical phenotype and prognosis of Bothnia dystrophy.

To analyze the retinal function electrophysiologically at different ages and stages of Bothnia dystrophy.

To evaluate the effects of prolonged dark adaptation on dark adaptometry and electrophysiological responses, and to gain a better understanding of the biochemical processes, and the pathogenesis in Bothnia dystrophy.

To screen for additional mutations in patients heterozygous for the R234W mutation in the *RLBP1* gene, and describe the phenotype.

To describe the prevalence and area of distribution of Bothnia dystrophy phenotype.

To assess the relationship between objective tests of visual function and vision-related quality of life in patients affected with BD disease

PATIENTS AND METHODS

PATIENTS

Paper I

Inclusion criteria for the seven families in this study were ophthalmologic records of retinal disease showing early onset night blindness, fundus appearance similar to RPA, macular degeneration, and lack of for RP typical bone spicules in peripheral retina.

Paper II

In the second paper the twenty affected, from the first study all homozygous for the R234W mutation, were invited for ophthalmological and electrophysiological studies. Four additional cases were added in order to define the phenotype more precisely. In addition, five unaffected heterozygotes, related to the index cases, were examined, and three of these carriers were subject to electrophysiological examinations.

Paper III

A total of nineteen patients were included in this study. The patients represented all the available BD patients with recordable ERGs, including four new patients who had not previously been presented. Out of the 19 patients, nine of the younger patients were chosen for an evaluation of the effect of prolonged dark adaptation on the retinal function.

Paper IV

DNA from 121 individuals affected with ARRP, originating from northern Sweden, was tested for the presence of the R234W mutation. The analysis showed that 61, including already known cases, were homozygous and 10 were heterozygous for the R234W mutation. The next step of the investigation was an extended genetic analysis of the *RLBP1* gene, along with a thorough clinical evaluation in the heterozygous patients.

Paper V

The study included 49 patients affected with BD. The participants were invited to do objective visual function tests together with a visual function questionnaire.

METHODS

ROUTINE OPHTHALMOLOGICAL EXAMINATION

The examination included slit-lamp examination, biomicroscopy with detailed fundus examination. Photographs of the fundi were taken, and fluorescein angiography of the retina was performed in selected cases according to the routine of the clinic.

Visual acuity

Visual acuity (VA) was tested, using a Monoyers visual chart in paper I, II and IV, and the EDTRS chart in paper III, and V. VA is presented as logarithm of minimum angle of resolution (logMAR). The decimal VA was converted into a log scale using the method outlined by Holladay and Prager (Holladay et al, 1991). In paper V weighted logMAR visual acuity (WVA) summarizes the acuity data from both eyes of the patient in one value (Scott et al, 1998). To calculate WVA, the better eye was weighted 0.75, and the worse eye 0.25, and the average of the two values was calculated.

Visual fields

Kinetic perimetry was performed with a standard Goldmann perimeter with light stimulus using standard (II-4-e and V-4-e) objects in all affected patients with any measurable vision. In paper III the visual fields were examined before and after prolonged DA (24 h) in selected cases. In paper V, binocular visual field maps were produced by merging the monocular fields of each subject via the method described by Arditi (Arditi, 1988). The visual field areas were then measured and analyzed by a computer.

Color vision

Color vision was tested with pseudoisochromatic plates (Ishihara´s test for colour-blindness, 38 plate edition, 1988) and Lanthony´s New Colour rearrangement tile test in all cases that were able to participate.

Contrast sensitivity

Low contrast VA was tested, using a Sloan letter logarithmic translucent contrast chart (2,5% and 10%). Participants who failed to read the largest letters at 4 m were tested at 1 meter. The contrast sensitivity (CS), was scored as the total number of letters read correctly and transformed to logarithm of the minimum angle of resolution (logMAR) units. Summarizing the measurements of contrast sensitivity of both eyes, weighted contrast sensitivity (WCS), the same method as calculating WVA was used as mentioned above.

Dark- adaptometry

Standard dark adaptation

The course of dark adaptation (DA) was determined using a Goldmann-Weekers adaptometer. Standard preadaptation with 1018 Candela/m^2 illumination during 5 min was made. The time and intensity required for a patient to distinguish whether the test pattern placed in the center of the visual fields was vertical or horizontal were noted. A target size of 11 degrees was used. In paper II, 14 patients were tested binocularly, and recordings during 40 to 45 min were measured.

Prolonged dark adaptation

In paper III, the visual sensory thresholds to light were measured during an extremely prolonged DA (24h). Measurements were made every 2 minutes during the first hour, then after every half-hour up to twelve hours. The eye to be examined was patched and the contralateral, eye left uncovered. Twenty-four hours after the start of the adaptometry test the final visual sensory thresholds, of both the extremely prolonged DA eye (24h) and the contralateral, unpatched eye, were determined.

Electrophysiology

Electrooculography (EOG), and full-field, single flash and flicker, ERGs, including the oscillatory potentials (OPs), were recorded (UTAS-E 2000 LKC Technologies Inc) using Burian-Allen bipolar electrodes and according to the recommendations of ISCEV (International Society of Clinical Electrophysiological in Vision). The isolated rod responses were measured during standard dark-adapted conditions (20 min) using full-field white flashes of low intensity (24dB attenuation). The dark-adapted mixed rod- cone responses were obtained using stimulation with flashes of maximum intensity (0dB). The OPs were recorded in dark adaptation using an interstimulus interval (ISI) of 30 seconds and in response to stimulus flashes of maximal intensity. The cone responses were elicited in light adaptation (white background illumination; 480 lumen/m^2) using maximum flash stimulation. The flicker ERGs (30Hz) were recorded in light-adapted eyes with an averaging technique (n=10) and in response to maximum intensity flashes.

The amplitude of the negative a-wave was measured from the baseline and the positive b-wave was measured from the a-wave trough. The amplitudes of the OPs were measured from the baseline drawn between successive throughs of the wavelets. The oscillatory response was measured as the summed amplitudes of the individual oscillatory peaks. The peak times were measured from stimulus onset to the peak of the negative a-wave, the positive b-wave or the oscillatory peaks.

Self-reported Visual-Function of Life Questionnaire

The National Eye Institute Visual Functioning Questionnaire (NEI VFQ-25) was chosen to evaluate the patients' subjective visual ability. The NEI VFQ-25 was administrated to the participants. Weighted distance logMAR visual acuity (WVA), weighted logMAR low contrast visual acuity (WCS) and binocular visual field (VF) areas were calculated. Vision-Related Quality of life was assessed using the 25-item NEI-Visual Function Questionnaire (VFQ-25) and the overall composite score was calculated.

STATISTICAL METHODS

In paper III the mean values of the relative amplitudes of all patients were graphically illustrated and compared. The relative amplitudes of the different components of the standard DA full-field ERGs were then statistically analyzed using the nonparametric Wilcox paired-sample test. Statistical significance was defined as $p< 0.05$.

In paper V the associations between age, the objective visual function results (WVA, WCS, binocular VF areas II-4-e, and V-4-e), and the composite score of the NEI VFQ-25 questionnaire were examined using Spearman's correlation analyses. Partial correlation coefficients were also reported to control for the influence of multicollienarity.

Multiple linear regression analyses were used to determine the associations between VFQ-25 composite score and age, WVA, WCS, VF (II-4-e, and VF V-4-e) areas. The models were

adjusted for gender by creating a bivariate variable. We modeled age, WVA, WCS and VF-area data as continuous variables.

All statistical analyses were carried out using SPSS 10.0 (Statistical Package for the Social Sciences for MS Windows, SPSS Inc, Chicago, IL).

MOLECULAR BIOLOGY

Genotyping

Blood samples were collected from patients and relatives in tubes containing EDTA, and extraction of genomic DNA was performed as described by Balciuniene et al, 1995 (Balciuniene et al, 1995). Initial screening for linkage to candidate gene regions, to known loci for RP or other retinal genes, was performed by the analysis of microsatellite markers using polymerase chain reaction (PCR). Further genotyping and direct genomic sequencing were performed of the gene *RLBP1* encoding CRALBP, after the linkage analysis had been completed. Linkage analyses were performed using the LINKAGE computer package, version 5.10, under the assumption of an autosomal recessive gene with 95% penetrance and a population frequency of 0.0158.

To test for the presence of the R234W mutation, a PCR-based diagnostic method was developed. The C12225T mutation (numbering, according to Genbank accession number L34219) corresponding to R234W abolishes a recognition site for the *Msp*I restriction endonuclease. Digestion mix after cleavage with *Msp*I enzyme was separated on 3% agarose gel.

Mutation analysis

PCR fragments for the mutation analysis were obtained by amplification from genomic DNA. For initial mutation screening, single-stranded conformational polymorphism analysis (SSCP) and denaturing high performance liquid chromatography (DHPLC) (WAVE, Transgenomic) were performed. An automated sequencing system (ABI Prism 377 Genetic Analyser) was used for the direct sequencing of the promoter region. A set of primers allowing amplification of overlapping PCR products was used for subsequent sequencing in both directions.

Sequence deviation T2614C (numbering according to L34219) was also defined by RFLP analysis after cleavage of the PCR product with *Tsp*509I enzyme. T2614C transition abolishes the *Tsp*509I site, which made it possible to distinguish T and C alleles. PCR fragments amplified with primers rd5F and rd5R were digested with *Tsp*509I enzyme and separated on 2% agarose gel (SeaKem, BMA, Rockland, ME). Sequence deviations T1610C, T2627C and G3024A (numbering according to L34219) were also defined by RFLP analysis after cleavage of the PCR products with *Mwo*I, *Rca*I and *Bpm*I respectively. Positions C1658A and A2695G were tested by the ARMS-PCR method.

Allele frequencies for different poymorphisms were estimated in Chinese, Indian, Lithuanian, Finnish and Saami populations and compared to a control group of drafted men from Västerbotten County.

RESULTS AND COMMENTS

THE GENOTYPE

Linkage analyses initially showed positive lod scores with the marker FES, located on chromosome 15q26.1 close to the gene (*RLBP1*) for cellular retinaldehyde-binding protein (CRALBP). The tight linkage between the Bothnia dystrophy locus and genetic markers located within a short distance from the *RLBP1* gene suggested that a mutation in the *RLBP1* gene might cause the disease phenotype. The analyse of the *RLBP1* coding sequence revealed a homozygous C to T substitution that would lead to an arginine (R) to tryptophan (W) substitution at amino acid 234 (R234W) of the protein CRALBP. All patients included in the linkage study (Paper I) were shown to be homozygous for the shift, whereas all the parents of the patient and most siblings were heterozygous. No unaffected individuals were homozygous. The analysis of 125 individuals (92 anonymous blood donors and 33 unaffected control subjects, ophthalmologically examined) revealed 3 heterozygous individuals and no individuals homozygous for the mutation. This corresponds to a disease allele frequency of 0,012 (3/250) in Västerbotten.

DNA from 121 individuals affected with ARRP was tested for the presence of the R234W mutation (Paper IV). The analysis showed that 61 were homozygous and 10 were heterozygous for the R234W mutation. To exclude compound heterozygosity, in the heterozygous patients, mutation screening of the coding sequence was performed. Since no additional mutations were found, direct sequencing were used to verify the presence of sequence variations in the promoter region. It was found that all patients heterozygous for the R234W mutation have a T to C transition at position 2614 (T2614C), –517 from the transcriptional start site of the *RLBP1* gene. Restriction-endonuclease analysis using Tsp509I enzyme revealed that 60 patients homozygous for the (R234W) mutation were also homozygous for the T2614C polymorphism. Only one individual homozygous for the R234W mutation did not have the T2614C polymorphism (case 204:1). The frequency of the variant T2614C allele established on 242 control chromosomes from drafted young men from the same geographical area was equal to 0.05, which was slightly higher than in Finnish, Saami and Lithuaninan populations.

INHERITANCE AND DEMOGRAPHICS

Thirty-one affected belonging to eleven families and 30 affected single cases all were homozygous for the R234W mutation. Analysis of the pedigrees of the BD-families showed a recessive inheritance pattern. One family (fam. 010) presented pseudodominant inheritance with an affected mother, and two affected sons. The patients with BD were asked for the origin of their parents, and it appears that all carriers of the gene with only single exception have their origin in Västerbotten County (Figure 5).

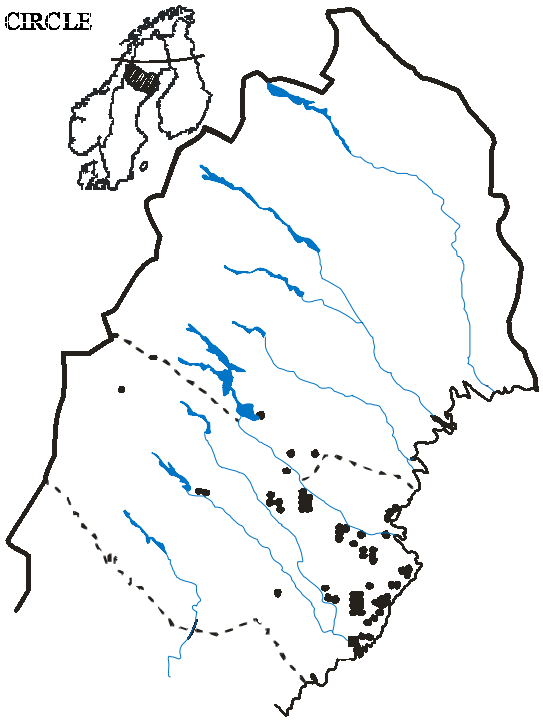

Figure 5: Map of Västerbotten County. Black dots show the birthplaces of parents to the patients. The hatched area in inset map shows the location of Västerbotten County in Scandinavia.

Age distribution

Age of patients	Year of birth	Number (%)	Female n (%)	Male n (%)
Year		61	36(59)	25(41)
8-17	1985-	3(5)	3	
18-27	1976-1985	5(8)	3	2
28-37	1966-1975	6(10)	4	2
38-47	1956-1965	7(11)	4	3
48-57	1946-1955	9(15)	5	4
58-67	1936-1945	8(13)	3	5
68-77	1926-1935	15(25)	9	6
78-87	1916-1925	6(10)	3	3
88-97	1906-1915	2(3)	2	

Table 1: Age distribution of patients affected with Bothnia Dystrophy (BD) in Northern Sweden, 2003.

THE PHENOTYPE

Routine ophthalmological examination

In total 49 of the 61 known BD patients ((49/61) homozygous of the R234W mutation) have been examined ophthalmologically in this study. In the evaluation of the patients heterozygous of the same mutation, eight of 10 patients were examined (8/10).

In one case nystagmus was observed from an early age. In this case VA was evaluated since childhood and never exceeded 0.2 to 0.3. One patient was treated with miotics because of glaucoma since the age of 32 years and developed cataract of nuclear type in her 60s. Examinations revealed no cataract of significance in any other subject and there were no indications of presenile cataract among BD patients in old medical records. Inspection of the cornea and iris as well as the vitreous body revealed no typical changes.

Fundus findings

Fundus examinations revealed no maculopathy of significance in younger individuals. However, retinal changes like salt and pepper could be observed in the mid-periphery in childhood. Typical subretinal white dots (RPA) were found in most cases and were first observed in the teens. Signs of maculopathy with central pigment deposits appeared in young adults and later areolar maculopathy developed. With increasing age, round retinal atrophies occurred paracentrally, and in the extreme periphery. In more advanced stages of the disease atrophy and widespread pigmentations with an appearance similar to bone spicules could occasionally be seen. Narrowing of the retinal vessels followed advanced retinal degeneration. However, the optic disk appeared well preserved except in the very high ages.

Angiographic findings

In the early arterio-venous phase there was a diffuse hyperfluorescense in the anatomic macular area and locally in the center of the fovea. Outside the arcades, and corresponding to the atrophic areas in the color fundus photograph, a general hyperfluorescense of granular type appeared. The hyperfluorescense indicates a gross atrophy of the pigment epithelium**.**

PSYCHOPHYSICAL FINDINGS

Visual acuity

In most cases the visual acuity (VA) shows a progressive decline with age, leading to legal blindness in the fourth decade of life (VA less than 0.05) following the WHO criteria. Low VA could occur as early as before the teens, or stay preserved to middle age in single individuals (Figure 6)

Refractive errors

All refractive errors were converted to spherical equivalents. Myopia of at least 0.5 D was observed in 12 of 49 cases (12/49), and hyperopic of at least 0.5 D in 16 of the 49 cases (16/49) when refraction was examined (Figure 7)

Visual fields

The visual fields were normal in all patients of young age. During the teens paracentral relative scotomas appeared. In young adulthood relatively deeper and larger scotomas, accompanied by a decrease in visual acuity, evolved. In the fifth decade absolute, extensive scotomas were present. In older patient’s only islets of the visual fields remained.

Color Vision

Color vision, tested with pseudoisochromatic charts, revealed a defect color sense (2-6 missed plates of 21 tested) in four of five affected children and teenagers. However, the test results using Lanthonys New Colour tiles were normal in these younger patients. In the early twenties the color sense of the patients was aggravated and abnormal trichromatism was

obtained using Lanthony´s New Colour test. In the adulthood, four of five tested with pseudoisochromatic plates revealed a grossly defect color sense (20-21 missed plates of 21 tested). In advanced stages of the disease it was no longer possible to evaluate the color vision due to poor VA.

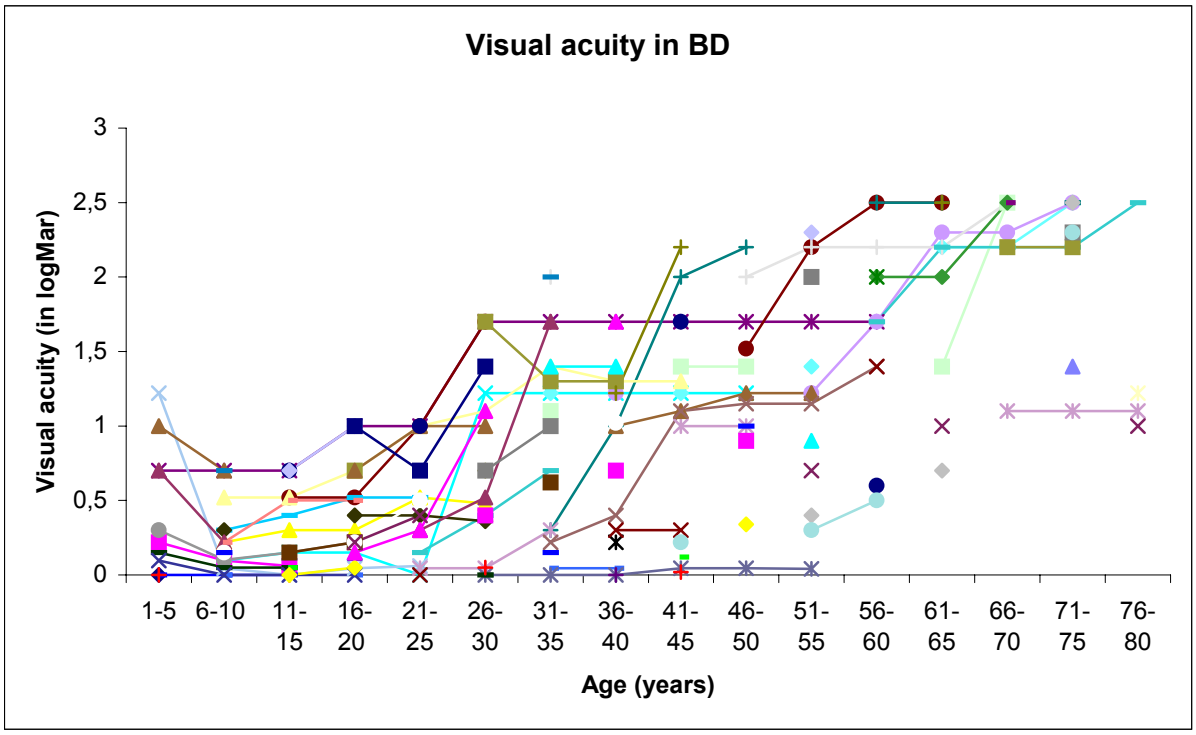

Figure 6: Visual acuity(VA) of cases affected with Bothnia Dystrophy observed in different ages. VAs are expressed in the logarithm of the minimum angle of resolution (logMAR).

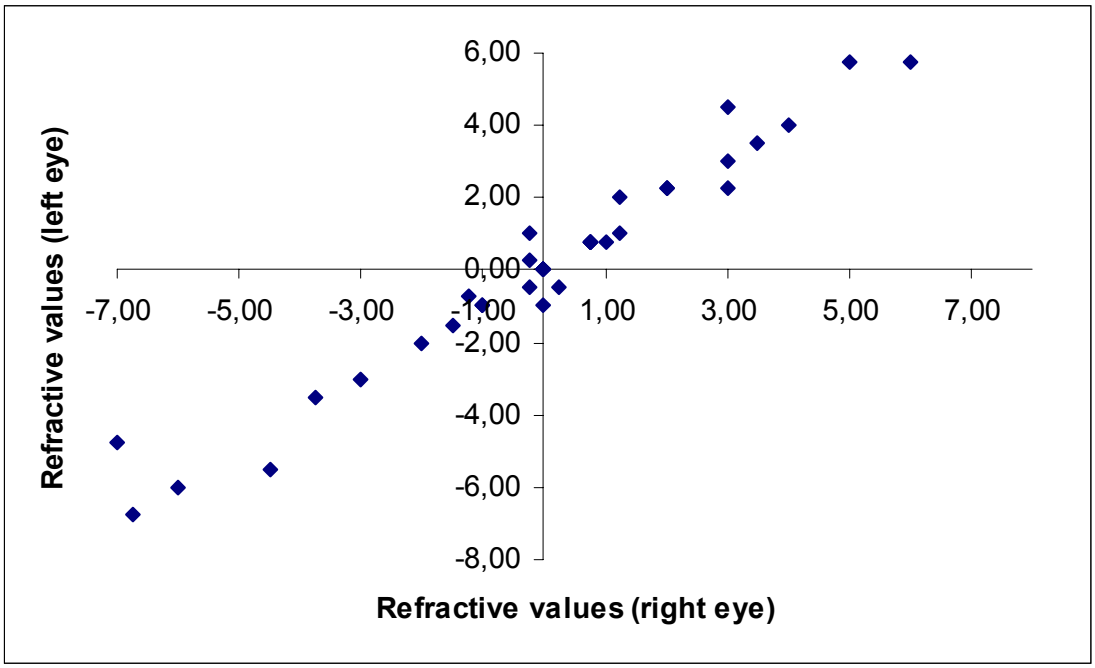

Figure 7: Spherical equivalents of refractive errors in BD patients.

Contrast sensitivity

Eighteen participants (18/49) were able to perform measurable results when testing the low-contrast VA (10%) chart. No patients were able to read the low-contrast VA (2,5%) chart in this group of patients tested.

Nyctalopia

In most individuals affected with BD there was a history of nyctalopia since two or three years of age.

ELECTROPHYSIOLOGICAL FINDINGS

Dark adaptometry

Standard dark adaptation

Dark-adaptometry tested during standard conditions showed abnormalities of both rod and cone adaptation in all 14 patients tested. Regardless of age, the rod function was severely affected and the cone adaptation was abnormal. In older affected cases there was an even more pronounced cone dysfunction. The dark-adapted final visual sensory threshold was elevated about four log units in all patients. In the most severe stages of the disease it was not possible to perform dark adaptometry.

Prolonged dark adaptation

In three older patients (33-47 years-old), prolonged dark-adaptometry of the patched single eye showed less than one log unit increase in sensitivity of the cones during the first hour. There was then a relatively rapid increase in sensitivity during the following four to six hours down to a level, about one half to one log unit above normal values in the two oldest patients and to normal in the youngest patient in this group.

The younger patients (12-21 years-old) showed similar results but also special features of recovery of sensitivity during extremely prolonged DA testing, compared to the first group of patients. There was a moderate, one log unit increase in sensitivity of the patched single eye during the first hour, followed by, in these cases, a plateau of recovery lasting up to four hours. Thereafter an almost linear increase of sensitivity occurred. They reached their final threshold levels of visual sensitivity not later than ten to twelve hours after the start of the dark adaptometry test of the patched single eye. Their final visual sensory thresholds were elevated about one half log unit, about one log unit and about two log units after the extremely prolonged DA (24h), respectively.

Electro-oculography

EOG was performed in eight cases, all of which presented subnormal Arden ratios (1.1-1.5).

Standard full-field ERG

The amplitudes of the rod isolated b-waves were subnormal or non-recordable in all patients. The peak times of the rod isolated b-waves were either within the normal range or prolonged. The amplitudes of the mixed rod-cone b-waves were subnormal or non-recordable. One single patient showed better-preserved rod-cone b-wave amplitude in one eye. The peak times of the mixed rod-cone b-waves were comparatively short in most patients but generally within the normal range in more advanced cases of the disease. The amplitudes of the mixed rod-cone a-waves were found to be within the normal range in some younger patients, subnormal in most adults and eventually non-recordable at old age. The peak time was within the normal range at

young age, but there was generally a prolonged peak time in adulthood. The amplitudes of the cone b-waves were better preserved and even within the normal range at the very young age. The peak times of the cone b-waves were generally within the normal range at a younger age, but prolonged in young adulthood and thereafter. The amplitudes and the implicit times of the 30-Hz flicker ERGs were within the normal limits, in most of the younger patients had normal amplitudes and implicit times. In early adulthood, subnormal and delayed flicker ERGs were recorded. At older ages low amplitude or non-recordable flicker ERGs were obtained.

The OPs were recorded as a routine procedure. The summed amplitudes of the individual oscillatory peaks showed subnormal values at young age, decreasing with age and were not recordable in patients older than 40 years.

Comparing the age-correlated relative mean values of the different components of the standard ERGs, the rod isolated responses and the mixed rod-cone b-waves were relatively more affected in patients with BD. The mixed rod-cone a-wave response and the OPs were relatively better preserved. The photopic responses (b-wave and 30 Hz flicker ERG) were best preserved, but on the average reached only 34 and 39 percent of the age-correlated normal mean values, respectively.

Prolonged dark adapted full-field ERG

Six patients were reexamined with full-field ERGs after one single eye had been DA for a prolonged period of time (10h) and the contralateral eye had been DA for a standard period (20 min). There was an increase of the rod isolated b-wave amplitudes of the prolonged DA eyes up to normal range in two patients (244:1 and 004:5, 19 and 21 years old). However, the b-waves were still delayed. No obvious increase in amplitude occurred in two patients with non-recordable or very low rod isolated b-waves (230:1 and 005:4; 19 and 20 years old). In two patients (013:6 and 013:5; 12 and 27 years old), unstable recordings were obtained, due to the interference of blink artefacts in response to stimulus light.

In most patients there was an increase in the amplitudes of the mixed rod-cone b-waves and/or the a-waves in the prolonged DA eyes. The peak times of the mixed rod-cone b-waves showed an increase with prolonged DA. The peak times of the a-waves were generally unchanged. No obvious change in amplitudes of the cone b-waves occurred after prolonged DA. Neither was there any significant difference in peak times of these components of the prolonged DA eyes as compared with their contralateral eyes.

A binocular comparison of the summed amplitudes of the OPs revealed after prolonged DA an increase in some patients with subnormal responses. One patient (244:1) reached values close to the lower limit of the normal range for her age.

HETEROZYGOUS AFFECTED INDIVIDUALS

It was not possible to detect any obvious differences in the clinical, phsycophysical or electrophysical outcome in the affected individuals, heterozygous for the R234W mutation compared to homozygous patients with BD.

VISUAL FUNCTIONS AND QUESTIONNAIRE

The mean age of the patients included was 49 years (SD=20.8; range 5-80). The age distribution of the patient group was as follows, 25% (Q1) of the patients were below 34 years of age, and 25%(Q3) of the patients were above 68 years of age. 31% of the BD patients had VA better than 20/60, 31% were visually impaired (20/400≤VA<20/60) and 39% were legally blind (<20/400) according to WHO definition of blindness (WHO, 1992). Eighteen

participants (18/49 (37%), predominantly in the younger ages (11-60 years) were able to perform measurable results, when testing the low-contrast VA (10%) chart. There are generally high levels of correlation between all variables studied (WVA, WCS, binocular visual field (VF) area (II-4-e and V-4-e objects (cm^2)), giving a strong hint of multicollinearity.

Partial correlation coefficients were also calculated. When the different variables were controlled for, there was a statistically significant correlation between, WVA and WCS, and WVA and VF area. The only statistically significant correlation with age was VF area (V-4-e object). Linear regression analyses showed that weighted visual acuity (WVA) was the strongest predictor for the total composite score of the questionnaire, and accounted for almost 70% of the variability of the composite score of the questionnaire, ie. the patients' subjective visual function. Our analyses show a significant correlation between each of the objective visual functions studied and subjective visual function. Linear regression analyses showed that all measurements of visual function tested, showed a statistically significant association with the patients subjective VRQL. As expected due to the progressive maculopathy present in the phenotype BD, age was also statistically associated with the composite NEI-VFQ-25 score.

DISCUSSION

A large number of RP patients in Västerbotten County, Sweden, were found to have a distinctive and unique clinical phenotype named Bothnia dystrophy (BD). Bothnia dystrophy is shown to be associated with the R234W mutation in the *RLBP1* gene localized on chromosome 15q26, coding for cellular retinaldehyde-binding protein (CRALBP). The affected protein is expressed both in the RPE and the Müller cells in the retina, two cell types bordering the proximal and distal ends of photoreceptors.

Severe night blindness is present from early childhood with elevated thresholds of dark-adaptation early in the course. Symptoms of defect macular function with a decrease of visual acuity appear in early adulthood. A poor low contrast VA was recorded in younger patients. The poor results when testing low-contrast VA might be associated with an early detection of foveal visual dysfunction in the BD phenotype. These results confirm earlier observed poor low-contrast VA in the RP group (Spellman et al, 1989). As the disease progresses, the retinal fundus shows irregular white spots in a central and parafoveal pattern along the arcades. With older age these white spots diminish and central areolar maculopathy develops. Round circular atrophies are recorded paracentrally and in the extreme periphery. These atrophies are reproducible in visual field tests. Hypopigmentation of the RPE, as revealed by fluorescein angiography, was found rather early in BD.

In order to evaluate the visual disability among BD patients, a study on the visual function was performed. The WVA had the strongest association with subjective visual function of BD patients, and there was a considerable loss of subjective and objective visual function with increasing age. In this group of patients no statistically significant differences between males and females was shown when comparing different VA-levels, WVA, WCS and VF areas, and composite score from the questionnaire. The females were younger than the males in this study, which might explain their better performance with the visual function tests and the VRQL composite score. Assessment of subjective visual function on patients with retinal disorders has been studied previously. Reports regarding typical RP patients have shown both the VA and VF area to correlate with patients´ self-assessments measured by a questionnaire. (Szlyk et al, 1997; Turano et al, 1999). The VF areas, analyzed monoculary, were notably smaller in the patients selected by Szlyk et al 1997, compared to BD patients. The larger VF areas measured in the present study indicate that BD patients represent a different setting from typical RP patients. We might therefore have another impact with the VF area results on VRQL in this RP group investigated. This may indicate a need for a different battery of visual tests when estimating patients' VRQL in different phenotypes of RP.

The electrophysiological results show that in BD there is a compromised rod and cone function, indications of a disturbed neuronal function of the inner retina, and a likely dysfunction of the Müller cells. Secondly, the findings indicate that there is a prolonged synthesis of photopigments, a retardation of the visual process in the RPE, and likely a loss of retinal photoreceptor cells starting at a relatively early age in BD.

In younger ages there was an improvement of the rod isolated and DA mixed rod-cone responses induced by prolonged DA of the retina. These findings indicate that the disturbed functions are reversible to some extent in the early phase of the BD disease, and this seems to apply both to outer as well as inner retinal functions.

Prolonged DA in BD patients, showed an extremely slow DA reaching steady state within 5 to 12 hours. In the younger patients apparent plateaus of recovery in the dark were observed and lasting for about 1 to 4 hours. It may be speculated that this plateau represents an effect on the removal of bleached pigments, which have been suggested to occur in other retinal visual cycle diseases (Cideciyan et al, 1997). The final visual sensory thresholds after 24-h-

DA had not returned to normal in most patients examined. This could well correlate to a disturbance in the normal function of CRALBP, and the process of regeneration of 11-*cis* retinal in the RPE. Slowed dark adaptation and extremely delayed regeneration of visual pigments have been reported in visual cycle diseases like vitamin A deficiency and Sorsby's fundus dystrophy (Kemp et al, 1988; Steinmetz et al, 1992). The presence of a prolonged cone plateau and a transitory rod plateau of recovery were observed in these disorders (Cideciyan et al, 1997). Recently a mutation in the visual cycle gene, 11-*cis*-retinol dehydrogenase (RDH5) gene, has been described. Its phenotype presents with fundus albipunctatus, and has been shown to cause a delayed rod-cone break and a final rod mediated threshold not to be reached until about 5 hours following full bleaches (Cideciyan et al, 2000).

In the present study, only one patient was found to have a complete recovery of rod function, measured psychophysically after prolonged DA. Accordingly, the true electrophysiological response in BD does not seem to show until a prolonged DA is used. However, no full restitution of the electrophysiological function (amplitude and timing) of the photoreceptors (rods, and cones) in the outer retina nor of inner retinal function was found in any BD patient in the present study. This might be due to the fact that the DA time was comparatively short (10h). Alternatively, the affected CRALBP may result in a relatively early, non-reversible, deteriorating damage and dysfunction of the Müller cells, the pigment epithelial cells, and degenerative changes in the photoreceptor cells.

As morphologic studies in humans are missing, further electrophysiological studies in the future seem to be necessary to fully understand the visual process and function in Bothnia dystrophy.

The migration of people and patients seems low and stable in Västerbotten County. Family pedigrees are known back to the 18th century due to earlier studies in this area. This gives us a unique possibility to trace the affected with RP and other retinal dystrophies, which is not an easy task. Samples from 121 patients with ARRP from Northern Sweden were screened for the R234W mutation. Sixty-one patients were shown to be homozygous and another 10 individuals were heterozygous for the R234W mutation and affected with the BD phenotype. All 71 patients originate predominately from Västerbotten County. We have found an overall prevalence of 1:2000 of non-syndromic RP including AD, AR and x-linked inherited RP in Västerbotten County. The patients affected with BD account alone for a prevalence of 1:3600. Hence there is a prevalence of RP, excluding BD, of 1:5300 in the population in Västerbotten County (2003). These findings should be compared to studies from Maine (USA) where patients affected with non-syndromic RP show a prevalence of 1:5193 (Bunker et al, 1984). Studies from Birmingham (UK) showed a prevalence of 1:4869 RP patients (Bundey et al, 1984), and in Denmark, where the prevalence of RP is reported to be 1:3943, including Usher, congenital amaurosis of Lebers, choriodermia, gyrate athrophy and Bardet-Biedl) (Haim et al, 1992).

The genetic analyses showed that patients heterozygous for the R234W mutation were also heterozygous at position –517 from the transcriptional start site of the *RLBP1* gene (T2614C) located in the promoter region of the *RLBP1* gene. The R234W homozygous patients tested for this observed inconsistency were shown to be homozygous for the T2614C polymorphism with one exception. Homozygosity for the polymorphism T2614C in the *RLBP1* promoter segregated with the BD disease. In the majority of BD patients, the T2614C and R234W are present on both alleles, but not in their unaffected relatives or controls. The presence of T2614C variant does not seem to result in a more severe phenotype.
The appearance of R234W heterozygotes among BD patients led to the assumption of compound heterozygosity, which was also reported by Morimura and Eichers (Morimura et

al, 1999; Eichers et al, 2002). However in spite of 10 patients heterozygous for the R234W, compound heterozygosity could not be confirmed. Therefore an alternative inheritance hypothesis, e.g. digenic or triallelic inheritance, known in Bardet-Biedl syndrome, a Mendelian recessive disorder, might be taken into account (Katsanis et al, 2001a).

Besides the R234W mutation responsible for BD other mutations in the (*RLBP1*) gene associated with retinal degenerations have been described. For example, one mutation (R150Q) in exon 5 of the *RLBP1* gene has been found in three patients from India (Maw et al, 1997), and in patients from Saudi Arabia (Katsanis et al, 2001b). Three additional mutations in the *RLBP1* gene have been reported in two patients belonging to small families of European ancestry (Morimura et al, 1999). Two splice junction mutations, one of which was reported by Morimura and collaborators in 1999, have been found in six Newfoundland pedigrees (Eichers et al, 2002). The phenotypes of these mutations all presented funduscopic findings with whitish fleck-like lesions like in RPA, peripheral degenerative changes and maculopathy. Thus, the BD phenotype does not seem to obviously differ in expression from other *RLBP1* mutations associated diseases, neither was there any difference in progression in BD compared to these four mutation phenotypes. However it cannot be excluded that other mutations or combination of mutations in the *RLBP1* gene could present other clinical phenotypes, such as a more typical RP type associated with or without RPA. A variation in the clinical phenotype has been reported in other genes of retinal dystrophies for example the ABCR and the RDS/peripherin gene (Weleber et al, 1993; Apfelstedt-Sylla et al, 1995; Klevering et al, 1999).

CRALBP is known to function as a carrier protein of retinoids in the RPE. Recently, studies on the retinoid binding properties of different CRALBP mutations have been performed. Results of mutation studies show that R150Q, and M225K mutations abolish, and that the R234W mutation tightens the retinoid binding. These mutations seem to impair CRALBP function in the visual cycle as an 11-*cis*-retinol acceptor and as a substrate carrier. It is not completely understood if only disturbed binding properties of the CRALBP protein lead to retinal disease or if the protein expression should be considered a possible cause of retinal malfunction and degenerative processes in Bothnia dystrophy (Golovleva et al, 2003).

CONCLUSIONS

- A missense mutation in the *RLBP1* gene is the cause of Bothnia dystrophy. The responsible gene was mapped to 15q26, coding for the cellular retinaldehyde-binding protein (CRALBP). Subsequent analysis showed that all affected patients in the initial study were homozygous for a C to T substitution in exon 7 of the *RLBP1* gene, leading to the missense mutation R234W.
- Bothnia dystrophy is a unique retinal dystrophy belonging to the rod-cone dystrophies with a high prevalence in northern Sweden. Patients affected with BD, typically show night blindness from early childhood. In young adults retinitis punctata albescens in fundus can be observed, followed by macular degeneration, and a decrease in visual acuity leading to legal blindness predominantly in early adulthood. Dark adaptometry and electrophysiologic testing showed a loss of rod and cone function.
- The low or non-detectable rod-isolated b-waves in the ERG indicate an early reduced or even lost rod photoreceptor function during standard DA conditions. Mixed rod-cone a-waves were found to be comparatively and significantly better preserved with age than the amplitudes of the mixed b-wave responses. This reflecting the less impaired function of the photoreceptors in the outer retina compared to the glial Müller cell response. The photopic responses were relatively better preserved during standard DA conditions, and reduced oscillatory responses in the BD patients at all ages indicate a comparatively early disturbance of the neuronal function in the inner retina.
- Using prolonged dark adaptometry, and prolonged dark adaptation (DA) full-field electroretinograms (ERGs), a gradual increase in retinal sensitivity to light and an improvement of the ERG components occurred. The findings indicate a prolonged synthesis of photopigments, retardation of the visual process in the retinal pigment epithelium (RPE), and a loss of retinal cells, probably starting at a relatively early age in BD.
- In Northern Sweden 61 patients, homozygous, and 10 patients, heterozygous for the R234W mutation, have been identified, predominately originating from Västerbotten County. Both groups presented a similar phenotype. No additional mutations in the *RLBP1* gene were identified in the heterozygous patients.
- The overall prevalence of non-syndromic RP including AD, AR and x-linked inherited RP is 1:2000 in Västerbotten County. The patients affected with BD alone account for a prevalence of 1:3600. Hence there is a prevalence of RP, excluding BD, of 1:5300 in the population in Västerbotten County (2003).
- Finally a strong relationship is found between objective tests of visual function, and patient perceived VRQL as assessed by a questionnaire, in patients with BD. WVA was the strongest predictor of self-reported experience of total visual function in BD patients.

SJUKDOMSYTTRING OCH FÖRÄNDRINGAR I ARVSMASSAN VID NÄTHINNESJUKDOMEN BOTHNIA DYSTROFI

POPULÄRVETENSKAPLIG SAMMANFATTNING

Det finns olika varianter av ärftliga sjukdomar i ögats näthinna, och patienter kan ha mycket skiftande symptom och uttryck av sin sjukdom. Dessutom kan samma sjukdom uttryckas på varierande vis hos olika individer. Ett samlingsnamn, retinitis pigmentosa (RP) finns för sjukdomar med nattblindhet och skador på synfältet. De kliniska varianterna är många, allt ifrån att man föds gravt synskadad till att man får en lätt synnedsättning i hög ålder. Under senare år har olika forskargrupper visat att olika RP typer är kopplade till specifika ändringar i arvsmassan som finns i varje cellkärna. Proteiner som berör näthinnecellernas funktion och överlevnad blir därför felaktiga vilket i sin tur leder till förtvining av näthinnan. Forskningsresultaten är ett stort steg mot förståelsen av sjukdomsprocessen trots att många frågor kvarstår. Vid institutionerna för oftalmologi och genetik har vår grupp bedrivit forskning som syftar till upptäckten och ökad förståelse av olika ärftliga näthinnesjukdomar i vår norra landsdel.

I decennier har en näthinnesjukdom med ett speciellt utseende och uttryck hos de drabbade varit känd av ögonläkare i Västerbotten. Flera fall har misstänkts höra till denna grupp av näthinnesjukdom, men lite kunskap fanns. Denna avhandling beskriver denna specifika näthinnedegeneration, Bothnia dystrofi, dess sjukdomsuttryck, kliniska och elektrofysiologiska fynd och de bakomliggande genetiska orsakerna. Vi har även undersökt hur patienterna själv uppfattar sin synfunktion och bästa sätt att värdera detta kliniskt.

I första delarbetet beskrivs hur den specifika genetiska förändringen lokaliserats till en gen på kromosom 15. Tidigare forskning har visat att denna gen kodar för ett bärarprotein lokaliserat i näthinnans pigmentepitel och stödjeceller. Proteinet deltar i omsättningen av vitamin A. Utredning av släktskap visade också att sjukdomen ärvs recessivt, dvs att det krävs två anlag för att få sjukdomen och att föräldrarna inte är drabbade.

Andra arbetet visar hur sjukdomen uttrycks med nattblindhet från barndomen med påverkad mörkertillvänjning. En tidig nedsättning av synskärpan i ungdomen relaterat till synfältsbortfall, färgblindhet och förändringar i det elektrofysiologiska svaret från drabbade sinneceller i näthinnan.

Det tredje arbetet utgörs av en fördjupad elektrofysiologisk studie. Denna visar att patienterna har en uttalad förlängd mörkertillvänjning och påverkan av olika celler i näthinnan. Förbättrade elektofysiologiskt svar vid förlängd mörkertillvänjning tyder på en bevarad om än nedsatt funktion av det sjuka proteinet.

I det fjärde arbetet kartläggs sjukdomens utbredning i Norrland. Flertalet patienter visar sig härröra från Västerbotten. Ett drygt 70 tal patienter återfanns med Bothnia dystrofi, vilket talar för en unik patientgrupp i denna landsdel. Fördjupat genetiskt arbete talar för att ytterligare genetiska faktorer, förutom den upptäckta mutationen, kan påverka insjuknandet i sjukdomen.

Det femte och avslutande arbetet visar hur patienterna egen subjektiva synförmågan avtar med åren och hur detta relateras till olika objektiva kliniska test av synfunktion.

Sammanfattningsvis har dessa fynd förbättrat möjligheterna för en tidig diagnos av Bothnia Dystrofi samt att ökande kunskaper om den bakomliggande orsaken till denna näthinnesjukdom ger möjlighet till framtida studier. Aktuell internationell forskning visar att sjukdomar som uttrycks i näthinnans pigmentepitel, såsom Bothnia Dystrofi, sannolikt är tillgängliga för terapeutiska åtgärder i framtiden.

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