Ocular Phenotype of Bothnia Dystrophy, an Autosomal Recessive Retinitis Pigmentosa Associated With an R234W Mutation in the \textit{RLBP1} Gene

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\textbf{Objective:} To describe the phenotype of Bothnia dystrophy, an autosomal recessive retinal dystrophy with an R234W mutation in the \textit{RLBP1} gene encoding cellular retinaldehyde-binding protein.

\textbf{Design:} Medical records were reviewed retrospectively. Ophthalmologic examination, including kinetic perimetry and, in selected cases, adaptometry, color vision tests, fluorescein angiography, and electrophysiologic studies, was performed. The study included 24 individuals, all homozygous for an R234W mutation in the \textit{RLBP1} gene.

\textbf{Results:} Patients typically show night blindness from early childhood. In young adults, retinitis punctata albescens was observed, followed by macular degeneration and a decrease in visual acuity that led to legal blindness in early adulthood. Dark adaptometry and electrophysiologic testing showed an initial loss of rod function followed by a progressive reduction of the cone responses in older ages.

\textbf{Conclusions:} Bothnia dystrophy is a unique retinal dystrophy belonging to the rod-cone dystrophies and has a high prevalence in northern Sweden. Fifty-seven cases of Bothnia dystrophy have been diagnosed, indicating a prevalence as high as 1 per 4500 population in the geographic area studied. A defect ability of mutated cellular retinaldehyde-binding protein to bind retinoid probably explains the defect rod function followed by central and peripheral degeneration.

\textbf{Clinical Relevance:} Retinal dystrophies associated with other mutations of the \textit{RLBP1} gene, including retinitis pigmentosa of Bothnia type, might account for a considerable number of cases of autosomal recessive retinitis pigmentosa in other geographic areas as well.


ETINITIS PIGMENTOSA (RP) is a group of inherited retinal disorders with a considerable variation in phenotypic expression and genetic background. Typical signs of the disease are night blindness and progressive loss of the peripheral visual field, 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). Examination of records for patients with RP in the Västerbotten County in northern Sweden has shown an accumulation of cases with a unique phenotype of RP named Bothnia dystrophy. Bothnia is the region in northern Sweden west of the Gulf of Bothnia, historically known as Bothnia Occidentalis. Affected individuals show night blindness from early childhood, with clinical features consistent with retinitis punctata albescens (RPA) and macular degeneration. The genetic defect that causes Bothnia dystrophy was recently shown to reside in the \textit{RLBP1} gene mapped to chromosome 15q26, encoding the human cellular retinaldehyde-binding protein (CRALBP). The CRALBP has been localized in retinal pigment epithelium (RPE) and Muller cells of the retina, ciliary body pigment epithelium, outer epithelium of the iris, cornea, and optic nerve, and the pineal gland. In the pigment epithelium, 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 consequently recycled back to the outer segment of photoreceptor cells of the retina. In vitro studies indicate that the presence of CRALBP diminishes the esterification and enhances oxidation of 11-cis-retinol. Patients affected by Bothnia dystrophy are homozygous for a C-to-T transition in exon 7 of the \textit{RLBP1} gene, leading to an arginine-to-tryptophan substitution at position 234.
SUBJECTS AND METHODS

SUBJECTS

Twenty patients from Västerbotten County were initially included in this study, and genetic analysis confirmed that all the affected individuals were homozygous for the R234W mutation in the RBP1 gene. 1 During the last year, new cases of Bothnia dystrophy were diagnosed using a polymerase chain reaction (PCR) assay. In December 1999, the number of cases homozygous for the mutation was 57. Clinical data from 4 additional cases were therefore added to define the phenotype more precisely. In addition, 3 unaffected heterozygotes, related to the index cases, were examined, and 3 of these carriers were subject to electrophysiologic examinations. The research was performed according to the Declaration of Helsinki and was approved by the local ethical committee.

GENETIC ANALYSIS

Extraction of genomic DNA was performed as described by Balciuniene et al. 13 Amplification of exon 7 was done by PCR, 1 using nonradioactive primers. Approximately 100 ng of PCR product was subsequently incubated with 0.5 U of MspI restriction enzyme (Boehringer-Manheim, Mannheim, Germany) at 37°C for 3 hours. The restriction endonuclease products were analyzed on 2.5% agarose gel (SeaKem; FMC Bioproducts, Rockland, Me) according to standard procedures. We took advantage of the fact that the C-to-T transition (C12225T) (GenBank account No. L34219) in exon 7 eradicates a MspI site. All known non-syndromic patients with RP in Västerbotten County were screened for the mutation.

CLINICAL AND FUNCTIONAL INVESTIGATIONS

After retrospective review of medical records of all cases, the patients were invited to undergo a complete medical eye examination performed by one of us (M.S.I.B.). Visual acuity (VA), using a Monoyers visual chart, was tested. Previous results of VA measurements were collected from available medical records, opticians, and centers for visually disabled. All refractive errors were converted to spherical equivalents. The 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. 14 The range of VA includes counting fingers, hand motions, and light perception. For VA less than counting fingers 0.5 m, the following arbitrary logMAR values were used: counting fingers in front of the eye, logMAR 2.2; hand motions, logMAR 2.3; and light perception, logMAR 2.5. 15

Slit lamp examination, biomicroscopy, and detailed fundus examination were performed. Visual fields were tested in a Goldmann perimeter using standard objects in all affected patients. Photographs of the fundi were taken, and photorecordings studied. Fluorescein angiography of the retina was performed in selected cases, and previously performed angiograms were analyzed. The course of dark adaptation was determined using a Goldmann-Weekers adaptometer. Color vision was tested with pseudospectral plates (the Ishihara test for color blindness, 38 plate edition, 1988) and the Lanthony New Colour rearrangement tile test in all cases who were able to participate. Electrooculography and full-field, single-flash, and flicker ERGs were recorded (UTAS-E 2000; LKC Technologies Inc, Gaithersburg, Md) using Burian-Allen bipolar electrodes and according to the recommendations of the International Society of Clinical Electrophysiological Vision. In dark-adapted conditions, rod responses were recorded using full-field white flashes of relatively low intensity (24-dB attenuation). Mixed rod and cone responses were obtained using stimulation with flashes of maximum intensity (0 dB). Cone responses were elicited in light adaptation to white background (480 lumen/m²) and using maximum flash stimulation (0 dB). Flicker ERGs (30 Hz) were recorded in light adaptation (480 lm/m²) using an averaging technique (n=10) and stimulation with maximum-intensity flashes. In 2 of the younger subjects, aged 8 and 15 years, recordings were obtained from only 1 eye because of poor cooperation. One young patient, aged 9 years, underwent ERG under general anesthesia.

RESULTS

GENETIC ANALYSIS

To correlate the clinical findings with the CRALBP genotype mutation, all individuals included in our study were tested for the presence of the R234W mutation. A PCR-based diagnosis method was therefore developed. The Bothnia dystrophy mutation alters a recognition site for the MspI, and the mutant allele can therefore be distinguished from the normal allele by MspI cleavage. The results of restriction endonuclease analysis confirmed that the mutation segregates with Bothnia dystrophy.

CLINICAL FINDINGS

In most cases the VA shows a progressive decline with age, leading to legal blindness in the fourth decade of life (Figure 1). However, one 50-year-old woman (case 065:2) with preserved VA in both eyes represents an exception. Four affected cases, examined since early childhood, never obtained a VA above 0.2 to 0.3. In one of these subjects (case 013:2), nystagmus was observed from an early age. In 4 cases, examined as children, there was an increase in VA as their refractive errors were corrected.

Fundus examinations revealed no maculopathy of significance in younger individuals (Table).
Clinical Psychophysical and Electrophysiologic Data of 24 Patients With Bothnia Dystrophy*

<table>
<thead>
<tr>
<th>Case No./Sex/Age, y</th>
<th>LogMAR Visual Acuity</th>
<th>Refractive Errors</th>
<th>Ocular Findings</th>
<th>Visual Fields</th>
<th>Electrophysiology</th>
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<tbody>
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<td>013:6/F/8</td>
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<td>−</td>
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<td>004:5/F/16</td>
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<td>−</td>
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<tr>
<td>013:5/F/23</td>
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<td>222:1/F/28</td>
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<td>AM</td>
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<tr>
<td>009:1/F/71</td>
<td>P/HM</td>
<td>−0.25/−0.25</td>
<td>AM</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* RPA indicates retinitis punctata albescens; EOG, electro-oculography; ERG, electroretinogram; CF, counting fingers; HM, hand motions; P, light perception; plus sign, present; minus sign, not present; SM, subtle mottling of the macula; AM, areolar atrophy of the macula; Np, not performed; Np (c), not performed because of poor cooperation; and E, nonrecordable.

Figure 1. Visual acuity of the better eye, expressed as logarithm of minimum angle of resolution (logMAR), related to age in all 24 patients. Symbols connected by a line are different measurements on the same patient. Star indicates single observation.
Retinal white dots (RPA) were found in most cases and were first observed in the teens (Figure 2A). Signs of maculopathy with central pigment deposits appeared in young adults (Figure 2B) and later areolar maculopathy developed (Figure 2C). With increasing age, round retinal atrophies occurred paracentrally and in the extreme periphery (Figure 2D). In more advanced stages of the disease, widespread pigmentations with an appearance
similar to bone spicules could occasionally be found (Figure 2D). Narrowing of the retinal vessels followed advanced retinal degeneration. However, the optic disc appeared well preserved in all cases examined. One patient (case 039:2) was treated with miotics because of glaucoma since the age of 32 years and developed cataract of nuclear type in her 60s. Ophthalmologic examinations revealed no cataract of significance in any other subject. The 5 unaffected relatives heterozygous for the mutation showed no clinical signs of retinal dystrophy.

**ANGIOGRAPHIC FINDINGS**

The fundus photograph and fluorescein angiogram of a young female patient is presented in Figure 3. A and B, respectively. The examination was performed 3 years after the electrodiagnostic evaluation. In the early arteriovenous phase, there was a diffuse hyperfluorescence 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 hyperfluorescence of granular type appeared. The hyperfluorescence indicates a gross atrophy of the pigment epithelium.

**PSYCHOPHYSICAL FINDINGS**

The visual field was normal in all patients of young age. The visual fields and the development of defects in one patient (case 005:3), registered during a period of 23 years, are presented in Figure 4. During the teens, paracentral relative scotomas appeared. In young adulthood, relatively deeper and larger scotomas, accompanied by a decrease in VA, evolved. In the fifth decade, absolute extensive scotomas were present. In older patients, only peripheral islets of the visual fields remained.

Color vision, tested with pseudoisochromatic charts, revealed a defect color sense (2-6 missed plates of 21 tested) in 4 of 5 affected children and teenagers. However, the test results using the Lanthony New Colour tiles were normal in these younger patients. In the early 20s, the color sense of the patients was aggravated and abnormal trichromatism was obtained using the Lanthony New Colour test. In adulthood, 4 of 5 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 because of poor VA.

Recovery of dark adaptation showed abnormalities of both rod and cone function in the 14 patients tested (Figure 5). In the younger patients, the rod function was severely affected or absent and the cone adaptation abnormal. The final dark-adapted sensitivity showed an elevation of about 4 log units. In older affected cases, there was an even more pronounced cone dysfunction. In the most severe stages of the disease, it was not possible to perform dark adaptometry.

**ELECTROPHYSIOLOGIC FINDINGS**

Electro-oculography was performed in 8 cases, all of which showed subnormal electro-oculography ratios (Table). Eighteen subjects underwent electrodiagnostic investigation including ERG. Representative full-field ERGs from 5 individuals of family 013 are shown in Figure 6. In the healthy woman (49 years of age), carrier of the mutation, the ERG showed normal recordings. In all affected cases (52, 42, 23, and 8 years of age), the rod and the rod-cone responses were severely reduced. There were no rod or mixed rod-cone responses recordable in older patients. The amplitudes of the cone B waves were subnormal and their implicit times prolonged. In the young girl (8 years of age), it was not possible to obtain reliable flicker ERG recordings. The amplitudes of the 30-Hz flicker ERGs were within normal limits in the 23-year-old woman but with increased implicit times. In the older relatives, the amplitudes of the 30-Hz
flicker ERG were severely reduced. In 3 unaffected relatives, heterozygous for the mutation, normal ERG findings were recorded.

COMMENT

In this report, a unique clinical phenotype of autosomal recessive RP, Bothnia dystrophy associated with the R234W mutation in the RLBP1 gene, is described. Severe night blindness is present from early childhood, with elevated thresholds of dark adaptation early in the course of the disease. Symptoms of defect macular function with a decrease of VA appear in early adulthood. Four cases were seen with low VA since childhood, which could be a manifestation of an early maculopathy. As the disease progresses, the retinal fundus shows irregular white spots within the retina 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. Fluorescein angiograms in early adulthood show a widespread hyperfluorescence in the retinal fundus, which indicates a grossly damaged RPE. In the early teens, most patients had the dark-adapted rod responses in ERG severely reduced. In all cases, rod-cone and cone responses were abnormal, although there seems to be a later involvement of the cone function. Early cases of Bothnia dystrophy may be hard to differentiate clinically from other variants of autosomal recessive RP until the typical appearance of RPA and maculopathy occurs.

The fact that RP of Bothnia type segregates with the R234W mutation in the RLBP1 gene is helpful in confirming clinical diagnosis. So far, the few published cases of RP associated with mutations in the RLBP1 gene have presented with a similar phenotype. Four affected siblings from a consanguineous family of Indian origin were homozygous for a mutation in exon 5 of the RLBP1 gene (R150Q) and progressed to legal blindness by their late 20s. Fundus examination showed macular degeneration and small white dots scattered over the whole fundus. It was shown that recombinant mutant protein (R150Q) was less soluble than wild-type protein and abolished binding to 11-cis retinaldehyde. Three additional recessive mutations have been reported in 2 patients belonging to small families of European ancestry, and those patients demonstrated a phenotype distinguishable from typical RP. One patient had a mutation located in exon 8, whereas another patient was a compound heterozygote with mutations in exon 6 and the intron-exon junction in intron 3. Clinical examination of these 2 cases also showed small yellow deposits at the level of the RPE across the fundus. In the older patient, there were round areas of atrophic RPE in the mid and far periphery, similar to our observations in Bothnia dystrophy.

Although a number of polymorphisms scatter over the whole RLBP1 gene, the mutations in patients with RP were found in exons 5, 7, and 8, creating proteins with affected C-terminal domain of the CRALBP. The significance of the C-terminal part was also shown by limited proteolysis, demonstrating remained retinoid-binding activity of the protein without the N-terminal part. Thus, only exons 5 to 8 might encode for motives responsible for ligand binding. The R234W mutation detected in patients with Bothnia dystrophy is in exon 7, and we may expect that mutant protein can significantly differ from the wild type. R234 is a conserved residue among orthologues to CRALBPs.
located close to Q210 and K221, which are shown to be part of the retinoid-binding pocket.17

A model explaining the phenotype is that the R234W mutation would lead to lacking ability to bind 11-cis-retinaldehyde, thereby preventing its regeneration and subsequently a loss of rod function as shown by electrophysiologic testing. This manifests as elevated thresholds of dark adaptation seen in all patients independent of duration of the disease. The ability of the mutant R234W protein to bind retinoid can be tested and will demonstrate whether R234 is involved in ligand binding. The progressive RPE and photoreceptor cell death manifested as central and peripheral degeneration might be explained by defect functional activity of the mutated protein that leads to toxic accumulation of retinoids in RPE.

At present we have diagnosed almost 60 cases of RP of Bothnia type associated with recessive mutation R234W in an area with a population of 257,000 inhabitants, and none of them had a diverging clinical appearance. However, it cannot be excluded that other mutations or combination of mutations (compound heterozygosity) in the RLBP1 gene could present with other clinical phenotypes, such as a more classic clinical picture of RP asso-

Figure 6. Dark- and light-adapted electroretinogram (ERG) responses from a healthy woman (49 years old) and her 2 affected sisters (cases 013:3 and 013:4) and 2 affected daughters (cases 013:5 and 013:6).
associated with or without RPA. A variation in clinical phenotype has been reported for patients with mutations in the ABCR gene. That change is associated with Stargardt disease but may also cause clinical pictures that resemble autosomal recessive RP. Similar conditions are well known from the RDS/peripherin gene.\(^1\)\(^2\)

Preliminary data indicate a frequency of patients with Bothnia dystrophy as high as 1 per 4500 population in Västerbotten County. The difference in prevalence is striking when compared with the material presented from Boston, Mass, by Morimura et al.\(^11\) In their study of 324 unrelated patients with RP or an allied retinal degeneration, a mutation in the RLBP1 gene accounted only for 3 cases. An analysis of blood samples from drafted young men from northern Sweden indicates a gene frequency as high as 1% to 2% in the population (I.G., unpublished data, 2000), which makes this condition a medical issue of local great importance.

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