Central Retinal Findings in Bothnia Dystrophy Caused by RLBPI Sequence Variation

Marie S. I. Burstedt, MD, PhD; Irina Golovleva, MD, PhD

Objective: To describe the central retinal findings early in the course of Bothnia dystrophy caused by the homozygous missense R234W sequence variation in the RLBPI gene.

Methods: In 8 young patients with Bothnia dystrophy (aged 9-34 years), high- and low-contrast distance visual acuity and visual fields were measured with Humphrey central (24-2) threshold testing and Goldmann perimetry. Central retinal thickness was measured with optical coherence tomography. Cross-sectional images were analyzed and a linear scanning protocol was applied to examine retinitis punctata albescence in the posterior pole.

Results: Affected visual acuity (4 of 8 cases) and poor low-contrast visual acuity (8 of 8 cases) were found. Significant foveal depression and visual field loss were evident with Humphrey threshold testing at all ages, and paracentral and central scotomata in the second decade of life advanced in adulthood as verified with Goldmann perimetry. Optical coherence tomography showed generalized retinal thinning in the central foveal, fovealmost ring diameter (Ø, 1 mm), and inner ring (Ø, 3 mm) areas in all ages, and early retinal thinning was found in the inferior areas of the outer macula (Ø, 6 mm). Foveal and extrafoveal thinning of the retinal layers and outer nuclear layer were found. Homogeneous retinitis punctata albescence changes were visualized in and/or adjacent to the retinal pigment epithelium–choriocapillaris complex with high reflectance.

Conclusions: In the RLBPI–Bothnia dystrophy phenotype, a loss of function and thinning of the central macula are found, indicating early damage of the cone photoreceptors in this disease of the visual cycle. Retinitis punctata albescence spots in the posterior pole are situated close to or in the retinal pigment epithelium–choriocapillaris complex.


A RELATIVELY LARGE NUMBER OF PATIENTS WITH AUTOSONAL RECESSIVE RETINITIS PIGMENTOSA, DENOTED BOTHNIA DYSTROPHY (BD), HAVE BEEN IDENTIFIED IN NORTHERN SWEDEN. THE DISORDER HAS BEEN ASSOCIATED WITH A MISSENSE SEQUENCE VARIATION, R234W, IN THE RLBPI GENE (OMIM #607475) AFFECTING THE CELLULAR RETINALDEHYDE-BINDING PROTEIN.1,2 THE BD PHENOTYPE IS CHARACTERIZED BY EARLY SEVERE NIGHT BLINDNESS, PROLONGED DARK ADAPTATION (5-12 HOURS), AND LATE RECOVERY (≤24 HOURS) OF THE RETINAL RESPONSES IN FULL-FIELD ELECTRORETINOGRAPHY.3 THE BD PHENOTYPE IS NOT ONLY AFFECTED BY THE BIOCHEMICAL TURNOVER OF RHODOPSIN IN THE VISUAL CYCLE, BUT PROGRESSIVE CENTRAL RETINAL DEGENERATIVE DISEASE IS ALSO PRESENT. MACULOPATHY AND CENTRAL FUNDS CHANGES WITH RETINITIS PUNCTATA ALBESCENS (RPA) ARE FOUND IN THE SECOND DECADE OF LIFE, WITH A DECLINE IN VISUAL ACUITY (VA) LEADING TO LEGAL BLINDNESS IN EARLY ADULTHOOD IN MOST PATIENTS WITH BD.4 VISUAL IMPAIRMENT IN PATIENTS WITH BD AFFECTS THEIR ABILITY TO PERFORM VARIOUS VISION-SPECIFIC FUNCTIONS OF EVERYDAY LIFE, AND THE DECLINE IN VA HAS BEEN FOUND TO BE THE STRONGEST PREDICTOR OF SELF-REPORTED ASSESSMENT OF TOTAL VISUAL FUNCTION IN PATIENTS WITH BD.5

To pursue the understanding of the important early pathological features and function of the central retina, psycho-physical measurements were obtained and central retinal imaging with optical coherence tomography (OCT) was performed to noninvasively determine the morphological features of the central retina and RPA findings in young patients with RLBPI-BD disease.

METHODS

PATIENTS

We included 8 young patients (aged 9-34 years) homozygous for the R234W sequence variation in the RLBPI gene. All individuals included in our study underwent testing for the presence of the R234W sequence variation.6,7
All 8 patients have been described in previous reports of the genotype and/or phenotype by our group.1,3-6

The studies have been approved by the Ethics Committee of Umeå University. Informed consent was obtained from all subjects, and the study followed the tenets of the Declaration of Helsinki.

### CLINICAL EXAMINATION

All patients with BD underwent a complete ophthalmological examination performed by one of us (M.S.I.B.).

#### Psychophysical Methods

Monocular VA of each eye was tested using the Early Treatment Diabetic Retinopathy Study (ETDRS) chart at a distance of 4 or 2 m. Monocular, low-contrast VA was tested using Sloan letter logarithmic translucent contrast charts (2.5% or 10% saturation) at a distance of 4 or 2 m. Lighting conditions were standardized using an ETDRS chart illuminator cabinet (No. 2425; Precision Vision, La Salle, Illinois). Visual fields (VFs) underwent monocular Humphrey Swedish interactive thresholding algorithm (SITA) standard 24-2 VF testing (Zeiss Humphrey Systems, Dublin, California), initiated by the foveal threshold value measurement (in decibels) according to the manufacturer’s instructions. The degree of the foveal threshold depression and the average elevation of depression of the overall field or loss in 1 part of the field (in mean deviations) was analyzed using the manufacturer’s software. The degree of significance outside the population was noted. The Goldmann perimeter (Haag-Streit, Berne, Switzerland) VFs were measured by an experienced assistant using the V-4-e, II-4-e, and 1-3-e targets.

#### Morphological Methods

The retinal thickness and structure were measured using OCT (Stratus OCT, model 3000; Carl Zeiss Meditec AG, Jena, Germany) after pupillary dilation in both eyes. According to the macular thickness map scan protocol, 6 consecutive macular scans of 6 mm in length, centered in the fovea (at equally spaced angular orientations) and including the fovea and adjacent perifoveal region (approximately 20°), were obtained. During each scan, the examiner (M.S.I.B.) confirmed that the image of the macula and fovea was centered with respect to the spot’s image on the fundus monitor. The central foveal thickness was defined as the mean thickness at the point of intersection of the radial scans. The foveal thickness was defined as the average thickness in the central 1000-µm diameter (ring diameter [Ø], 1 mm).7

### RESULTS

#### CLINICAL FINDINGS

Low distant VA was found in 4 of the 8 patients with BD (Table). All participants had poor results when they underwent testing with low-contrast VA charts (2.5% saturation), and 2 women (patients 6 and 8, aged 25 and 34 years) were unable to see any letters on the low-contrast VA charts used (2.5% and 10% saturation).

With Humphrey SITA standard 24-2 visual field testing of the foveal threshold, significant foveal depression (defined as P < .01) was found in 7 of the 14 eyes undergoing testing. The mean deviation of the Humphrey SITA standard 24-2 VF testing showed significant loss (P < .005) in all 14 eyes examined, indicating an overall
depression and/or significant loss of parts of the VF in BD (Table). Representative grayscale results of Humphrey SITA standard 24-2 VF testing are shown in Figure 1 (patients 1, 3, and 7, aged 9, 17, and 30 years, respectively). In the youngest patient, the VF testing revealed a depressed sensitivity pattern in the upper VF of both eyes (Figure 1A). More advanced sensitivity depression in the VF was found centrally in the 2 older female patients, and a pattern of spared areas in the lower part of the VF was noted (Figure 1B and C).

Goldmann perimetry was found to be unaffected in patient 1, who was the youngest (9 years old). Relative parafoveal scotoma and/or ring scotoma was found in the teenaged patients, and large, deep to absolute scotomata were found centrally in both eyes in patient 8, who was the oldest (34 years old).

FIGURE 1. Left and right visual fields obtained with Humphrey central (24-2) threshold testing using the Swedish interactive thresholding algorithm (Zeiss Humphrey Systems, Dublin, California) in Bothnia dystrophy. A, A 9-year-old girl (patient 1); B, A 17-year-old girl (patient 3); C, A 30-year-old woman (patient 7).

REPRESENTATIVE OCT IMAGES

Cross-sectional OCT images and corresponding retinal thickness maps in a 30-year-old control and in 3 patients with BD are given in Figure 3. In the control subject, the low-reflectance outer nuclear layer (ONL) peaks in thickness in the center beneath the foveal depression, and the ONL is visible to the periphery of the image. A third HRB is distinguishable just above the thicker high-reflectance RPE-choriocapillaris complex with a slight convexity in the center, possibly reflecting longer cone outer seg-

The mean central foveal thickness was 124 (10) µm, the mean foveal thickness (Ø, 1 mm) was 136 (9) µm, and the mean total macular volume was 6.1 (0.4) mm³ in the examined patients with BD. The corresponding figures for the control group were 176 (23) µm, 214 (25) µm, and 7.0 (0.1) mm³, respectively. Macular thickness measurements for each region in the patients with BD and age-matched normal controls are shown in Figure 2. The data from the normal controls were found to be well in accordance with those of previous studies. In the patients with BD, measurements of central foveal thickness, foveal thickness (Ø, 1 mm), and the inner ring of the retina (Ø, 3 mm) showed subnormal thickness at all ages. In the outer ring (Ø, 6 mm), generalized thinning was seen predominantly in the inferior regions of the OCT measurement, and a trend toward more preserved areas of retinal thickness of the superior and nasal regions was found in the examined patients with BD compared with normal controls. In all cases studied, the total macular volumes were low compared with those of the controls. Similar retinal thickness results were found in both eyes. We found thinning of the central foveal thickness across all ages of the young patients with BD examined. Although there was some variance between individuals, no obvious age trend was detected in the younger patients, but more notable thinning was found in the foveal area (Ø, 1 mm), the inner (Ø, 3 mm) and outer (Ø, 6 mm) ring areas, and the total macular volume of the oldest participant (patient 8, aged 34 years) compared with the younger patients with BD who were examined in this study.

FIGURE 2. Optical coherence tomography macular thickness measurements of 10 areas in patients with Bothnia dystrophy and normal control subjects. The central foveal, foveal (ring diameter [Ø], 1 mm), inner (Ø, 3 mm), and outer (Ø, 6 mm) ring superior (S), inferior (I), temporal (T), and nasal (N) areas are shown. The curve for normal controls is also given and includes limit lines that represent standard deviation.

The mean central foveal thickness was 124 (10) µm, the mean foveal thickness (Ø, 1 mm) was 136 (9) µm, and
ments in this region. The tomogram from the youngest patient with BD shows foveal thinning but still has a visible low-reflectance ONL throughout the image (Figure 3B). The third HRB is partially visible, with a slight convexity in the center of the fovea in the 2 younger patients with BD (Figure 3B and C). The tomogram of the older patient with BD (Figure 3D) shows thinning of the retinal layers in the center and periphery, with a partially absent low-reflectance ONL; no visible third HRB was found. In the examined patients with BD, no macular edema or cystic changes in any of the retinal layers were found.

VA, CENTRAL RETINAL THICKNESS, AND THIRD HRB

We compared VA with the central foveal thickness (15 eyes) and the foveal thickness (Ø, 1 mm) (16 eyes). The third HRB was evaluated and plotted. In 2 of the 16 eyes examined, the third HRB was absent, whereas 9 eyes had a partially distinct band and 5 eyes had an intact band. The results are illustrated in Figure 4. When we examined VA and central foveal thickness in young patients with BD, uniform central foveal thinning was found. The patients with the lowest VA results had the thinnest central foveal measurement and an absent third HRB. With better VA results, an intact third HRB was often seen (Figure 4A). Comparison of the VA and foveal thickness (Ø, 1 mm) showed a more obvious trend in which preserved retinal thickness resulted in better VA results and an intact third HRB (Figure 4B) in this comparatively small group examined.

MORPHOLOGICAL FINDINGS OF RPA

Retinitis punctata albescens was found in 11 of the 16 eyes examined. Signs of RPA were seen predominantly in the macula area and adjacent to the arcades, varying from single to multiple generalized white lesions scattered over the posterior pole of the retina. Figure 5A and B shows a binocular color fundus photograph of the 23-year-old man with multiple generalized RPA lesions (patient 5). The vertical line represents the scan traversing the retina at the temporal area of the macula between the arcades. In Figure 5C and D, 30-year-old patient 7 had a pseudo–macular hole appearance and more sparsely scattered RPA changes centrally and outside, adjacent to the arcades. Corresponding color images and grayscale OCT images of the right and left eyes visualizing the RPA changes in the retina are shown. These homogeneous, rounded, and highly reflective retinal changes could be seen adjacent to and in the high-reflectance RPE-choriocapillaris complex, present in the color and gray-
scale OCT images. Findings of thinning or compression in the ONL overlaying the RPA lesions were noted, and the RPE was found without obvious disruption, elevation, or detachment adjacent to the RPA lesions.

**COMMENT**

In retinitis pigmentosa of RLBP1-BD type, early visual impairment, measured using distant VA, was found. In all the younger case patients, an overall severe effect consisting of low-contrast VA and central VF loss was found. Generalized thinning of the central foveal, foveal, and inner (Ø, 3 mm) pericentral areas of the macula, reproducible in VFs, was present early in BD. Previous studies of retinal degenerative disease of more typical forms of retinitis pigmentosa have shown central retinal thinning and retinal thickening when measured by OCT. In BD, the outer macula area (Ø, 6 mm) showed thinning of the inferior part of the retina early in the course of the disorder, with more preserved areas of retinal thickening.
ness, predominantly of the superior part, in the young-est patients. In studies of Leber congenital amaurosis with sequence variations of the RPE65 gene, another retinal degenerative disease affecting the visual cycle, Jacobson and coworkers12 described prominent photoreceptor loss in the foveal and extrafoveal retina, even in the young-est patients studied (aged 6-17 years), with relative pres-ervation localized in the superior-temporal and temporal-pericentral retina and the ONL.

In BD, the cross-sectional morphological features visu-alized by OCT showed general thinning of the cell layers and a reduced ONL. In this comparatively small group of patients, a trend of foveal thinning and a less distinct third HRB were associated with more affected VA results.

In addition, the third HRB found in the younger pa-tients was less distinct in the younger adults. This band possibly represents the outer segment length of photoreceptors, predominantly the cones in the fovea.13,14,15 This finding indicates an effect on the cone photoreceptors and a possible degeneration of the outer segments of cones early in the course of BD. A previous study of a single young patient with a compound heterozygous (R103W/ R234W) sequence variation in the RLBP1 gene also re-ported decreased retinal thickness and degenerative signs in the outer retinal layer.13

A typical clinical expression of the BD phenotype is night blindness, which is severely affected dark adaptometry and absence of recordable scotopic response in full-field electroretinography with standard dark adap-tation time (ie, a primarily rod photoreceptor disease). However, a significant capacity for recovery of rod photoreceptor function with extremely prolonged dark adap-tation (24 hours) has been reported, in contrast to cone photoreceptor response that shows disturbed irreversible function and starts at a relatively young age in BD.3 The regeneration of rod function can be explained by con-tinuous but slow regeneration of rod photopigments occurring for at least 24 hours, and tightened retinoid binding properties of the mutant cellular retinaldehyde-binding protein may be involved in BD.14 The foveal thinning found in this study, suggesting early degeneration of cone photoreceptors in BD, may be explained by the cones also incorporating 11-cis retinoids derived from the rod and cone visual cycles in their visual pigments and by the fact that Muller cellular retinaldehyde-binding protein, possibly affected in BD, participates in the cone visual cycle in vertebrate phototransduction.15 In a study examining the visual cycle in the RPE65 and lecithin reti-nol acyltransferase knockout animal models, a key role for cell survival was found to be 11-cis-retinal bound to cone opsins, which are important for retinal protein sort-ing, transport, and targeting.16

Subtle white lesions of the fundus, or RPA, have pre-viously been found in several RLBP1 sequence vari-a-tions,15,17-21 and other progressive degenerative diseases, such as rhodopsin-related retinitis pigmentosa, have also been reported with such signs.22 This study shows that RPA lesions are often found on the posterior pole of young patients with BD and associated RLBP1 sequence varia-tion; however, not all young patients with BD have these lesions. The homogeneous, rounded, and highly reflect-ive changes were seen adjacent to and within the high-reflectance RPE-choriopapillaris complex. The localization and appearance of the RPA lesions resemble more commonly known retinal lesions such as drusen in age-related macular degeneration. This pathological process is known to be initiated by lipid peroxidase in the Bruch membrane or RPE lysosomes, causing RPE cell death.23,24 However, the RPA lesions in the young pa-tients with BD showed no obvious elevation, disrupt-ion, or detachment of RPE, and the central lesions were restricted in size without the confluence often found in age-related macular degeneration. Another known finding in age-related macular degeneration is that the accumu-lation of material between the retina and choriopapil-laris interferes with the exchange of nutrients and products close to the drusen, inducing RPE or neural retinal damage with overlaying photoreceptor cell layer thinning, predominantly affecting the photoreceptor outer segment.25-27 We found similar thinning or compression of the ONL overlaying the RPA lesions in this study, the cause of which could be the accumulation of products in the RPE because of higher affinity and impaired 11-cis-retinal release in the visual cycle in the BD phenotype.14,16,20

To conclude, in RLBP1-BD disease, thinning of the cen-tral foveal and foveal area of the macula is found in child-hood, with generalized thinning of the cell layers and low-macular volume increasing with age. Central thinning of the ONL and possibly of the cone photoreceptors may already be irreversible in the early course of the disease. In the study reported herein, changes to the posterior pole in the BD phenotype that we visualized with OCT were found to be homogeneous, highly reflective, rounded lesions and were located adjacent to or in the high-reflectance RPE-choriopapillaris complex.

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Correspondence: Marie S. I. Burstedt, MD, PhD, Depart-ment of Clinical Sciences and Ophthal-mology, University of Umeå, SE-901 85 Umeå, Sweden (Marie.Burstedt@ophthal.umu.se).

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