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Retinal morphology in patients with BBS1 and BBS10 related Bardet–Biedl Syndrome evaluated by Fourier-domain optical coherence tomography

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Abstract

Retinal dystrophy in Bardet–Biedl Syndrome (BBS) is caused by defective genes that are expressed within ciliated cells such as photoreceptors. The purpose of this study was to characterize and compare the retinal structure and lamination of two groups of patients, carrying mutations in BBS1 or BBS10. Eight patients with BBS (ages 11.9–28.5 years) and mutations in BBS1 (4/8) or BBS10 (4/8) were tested. A high-resolution hand-held probe Fourier-domain optical coherence tomography system (Fd-OCT) was used for retinal image acquisition. Macular scans were evaluated with respect to structure, retinal layering and photoreceptor integrity. Micro-structural in-vivo analysis showed abnormalities within retinal layers but preserved retinal lamination. Photoreceptor integrity was disrupted in all patients. Macular scans from patients with BBS10 mutations most often showed ‘deposits’ adjacent and anterior to Bruch’s membrane. Age, genotype and presence of macular changes did not correlate with the structural changes observed. Retinal dystrophy in BBS is reflected by major changes in the outer retinal layers. This is the first report of in-vivo micro-structural analysis of retinal layers in patients with BBS. Mutations in different BBS genes seem to be associated with similar micro-structural changes in retinal layers.

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Keywords: Bardet–Biedl Syndrome; BBS1; BBS10; Fourier-domain OCT; Retinal lamination

1. Introduction

Bardet–Biedl Syndrome (BBS)1, first described by Bardet (1920) and Biedl (1922), is defined as an autosomal recessive multi-systemic disorder. Cardinal features include digit anomalies, obesity, cognitive impairment, hypogonadism, kidney malformation or dysfunction and retinal dystrophy (Churchill, McManamon, & Hurley, 1981; Schachat & Maumenee, 1982). BBS is now thought of as a ‘ciliopathy’ with the underlying defects affecting the basal body of ciliated cells, such as the connecting cilium in the outer retina (Ansley et al., 2003; Ross et al., 2005). Twelve different genes have been associated with the disease, with BBS1 and BBS10 being the most frequently involved (Beales et al., 2003; Stoetzel et al., 2006).

The retinal dystrophy in BBS is progressive and shows variable severity. Retinal layer analysis using time-domain optical coherence tomography (OCT) in patients with homozygous or compound heterozygous mutations in BBS1 was performed by Azari et al. (2006). Definable lamination was observed with no extreme retinal thickening and a normal nerve fiber layer around the optic nerve for most of the 10 patients tested. No genotype–phenotype correlation or age-matched correlation was identified.

Fourier-domain OCT (Fd-OCT) permits faster retinal image acquisition with significantly higher axial resolution...
than commercial time-domain OCT systems (Wojtkowski, Leitgeb, Kowalczyk, Bajraszewski, & Fercher, 2002; Nassif et al., 2004). Application of Fd-OCT in patients with retinal diseases has provided an opportunity to delineate details of retinal structure and lamination (Gerth et al., 2007; Sun et al., 2007).

Here, we describe retinal structure characteristics using a custom high-resolution imaging instrument in younger patients with BBS1 or BBS10 mutations. We found preserved inner retinal layers but disrupted outer retinal structure without definable connecting cillum (inner/outer segment junction) in most of the patients studied. Retinal structure abnormalities were not correlated with either genotype, age or disease severity.

2. Materials and methods

2.1. Subjects

Patients diagnosed with Bardet–Biedl Syndrome carrying either a homozygous or compound heterozygous mutation in the BBS1 or BBS10 genes were recruited through the Ocular Genetics Clinic at The Hospital for Sick Children (Sick Kids) in Toronto, Canada. A comprehensive ocular assessment as well as a detailed phenotype review of systems; body mass index, kidney structure and function, liver function and digit anomalies were completed. Presence of cognitive impairment was assessed through parental interviews. Written informed consent and/or assent were obtained from all participants and/or their guardians. The project was approved by the Research Ethics Board at Sick Kids and conducted in accordance with the Tenets of Helsinki.

2.2. Ocular function and morphology assessment

The comprehensive eye evaluation included dilated fundus examination, visual acuity (VA), kinetic perimetry and full-field electoretinography (ERG). Best-corrected monocular distant visual acuity (VA) was measured on the backlit Early Treatment Diabetic Retinopathy Study Charts using a logMAR scale (Ferris, Kassoff, Bresnick, & Bailey, 1982). A VA of 'counting fingers' was converted into 1.85 logMAR (Schuiz-Bonsel, Feltgen, Burau, Hansen, & Bach, 2006). Monocular kinetic visual fields (VF) were performed on the Goldman perimeter using test target III-4e. VF area was measured as a solid angle using the VisFields 1.3.2. (Weleber & Tobler, 1986). Full-field electoretinograms (ERG) were recorded according to the ISCEV standard (Marmor, Holder, Seeliger, & Yamamoto, 2004) and compared with age-matched control data.

2.3. Fourier-domain optical coherence tomography

In-vivo high-resolution retinal image acquisition was performed using a Fourier-domain high-speed, high-resolution optical coherence tomography (Fd-OCT) system (axial resolution: 4.5 μm; acquisition speed: 9 frames/second, 1000 A – scans/frame) constructed at the UC Davis Medical Center (Alam et al., 2006; Zawadzki, Bower et al., 2005) with a sample arm replaced by a hand-held scanner (Bioptigen Inc. Durham, NC). Horizontal scans of 5 mm were obtained through the macular area. Images were post-processed as described in detail elsewhere (Zawadzki, Bower et al., 2005; Zawadzki et al., 2007).

Retinal layers were identified based on previously published data (Zawadzki, Jones et al., 2005) and compared with control data as shown in Fig. 1. We (Zawadzki, Jones et al., 2005) and others (Wojtkowski et al., 2005; Wojtkowski et al., 2004) have identified three distinct bands in the outer retina using high-resolution Fd-OCT, which are not visible with lower-resolution systems. Direct overlay of OCT and histological sections from the monkey retina (Macaca fascicularis), following correction for shrinkage, led to positive identification of the inner/outer segment junction corresponding to the connecting cilia (Anger et al., 2004). Similar results were found in the porcine retina (Gloesmann et al., 2003).

2.4. Mutational analysis

Mutation screening was performed for all coding exons and splice junctions of BBS1 (NM_024649) and BBS10 (NM_024685). Mutational analysis was performed by direct sequencing of purified polymerase chain reaction (PCR) products amplified from genomic DNA, using standard protocols. A complete list of primers used for PCR and sequencing is available upon request. Sequence changes were verified with family segregation, when the family was available. A minimum of 150 healthy control individuals were screened to assess the allele frequency of novel sequence changes.

3. Results

Eight patients (5 females, 3 males) between ages 11.9 and 28.5 years with mutations in the BBS1 (n = 4) or in the BBS10 gene (n = 4) were included in the study. Systemic features of patients studied are summarized in Table 1.

Results of ocular assessments are summarized in Table 2. Visual acuity ranged from 0.2 to 1.85 logMAR. Despite their younger age, most of the patients carrying mutations in BBS10 had more severely reduced VA and smaller visual field areas than patients with mutation in BBS1.
### Table 1
Genotype–phenotype Summary

<table>
<thead>
<tr>
<th>Case #</th>
<th>Gender</th>
<th>Gene</th>
<th>Mutation</th>
<th>Age (years)</th>
<th>Kidney anomaly</th>
<th>Liver anomaly</th>
<th>Digit anomaly</th>
<th>Limb #</th>
<th>Weight</th>
<th>Cognitive impairment</th>
</tr>
</thead>
<tbody>
<tr>
<td>2597</td>
<td>F</td>
<td>BBS1</td>
<td>M390R/M390R</td>
<td>20.6</td>
<td>No</td>
<td>No</td>
<td>Polydactyly</td>
<td>1</td>
<td>Overweight</td>
<td>Learning disability</td>
</tr>
<tr>
<td>2512</td>
<td>F</td>
<td>BBS1</td>
<td>M1V/M1V</td>
<td>28.5</td>
<td>No</td>
<td>No</td>
<td>Brachydactyly</td>
<td>NA</td>
<td>Obese</td>
<td>No</td>
</tr>
<tr>
<td>2301</td>
<td>F</td>
<td>BBS1</td>
<td>D8D/R483X</td>
<td>15.8</td>
<td>Yes</td>
<td>Yes</td>
<td>Polydactyly</td>
<td>4</td>
<td>Obese</td>
<td>Dev delayed</td>
</tr>
<tr>
<td>2293</td>
<td>M</td>
<td>BBS1</td>
<td>M390R/N524del</td>
<td>20.8</td>
<td>No</td>
<td>No</td>
<td>NA</td>
<td>NA</td>
<td>Obese</td>
<td>Dev delayed</td>
</tr>
<tr>
<td>2296</td>
<td>M</td>
<td>BBS10</td>
<td>C91W/A474fs483X</td>
<td>11.9</td>
<td>Yes</td>
<td>No</td>
<td>Polydactyly</td>
<td>4</td>
<td>Overweight</td>
<td>Dev delayed/Autistic</td>
</tr>
<tr>
<td>2621</td>
<td>M</td>
<td>BBS10</td>
<td>C91W/A474fs483X</td>
<td>13.6</td>
<td>Yes</td>
<td>No</td>
<td>Clinodactyly</td>
<td>2</td>
<td>Obese</td>
<td>NA</td>
</tr>
<tr>
<td>2213</td>
<td>F</td>
<td>BBS10</td>
<td>C91W/V707fs708X</td>
<td>13.5</td>
<td>Yes</td>
<td>No</td>
<td>Polydactyly</td>
<td>1</td>
<td>Overweight</td>
<td>Dev delayed</td>
</tr>
<tr>
<td>2294</td>
<td>F</td>
<td>BBS10</td>
<td>C91fs95X/R103fs110X</td>
<td>15.5</td>
<td>Yes</td>
<td>No</td>
<td>Polydactyly</td>
<td>4</td>
<td>Overweight</td>
<td>Dev delayed</td>
</tr>
</tbody>
</table>

1, novel mutation; Age, age at time of kidney and liver blood work assessment; Kidney anomaly, defined as either abnormal function as per electrolyte and elevated creatinine and urea levels and/or abnormal kidney ultrasound; Liver anomaly, defined as either elevated liver enzymes and/or abnormal liver ultrasound; Weight, based on percentile for body mass index-for-age; Obese: >95th percentile and Overweight: 85th–95th percentile; NA, not available; Limb#, refers to the numbers of limbs affected with a digit anomaly.

### Table 2
Ocular phenotype and OCT findings

<table>
<thead>
<tr>
<th>Case #</th>
<th>Gender</th>
<th>Age</th>
<th>VA</th>
<th>GVF</th>
<th>ERG (age at test)</th>
<th>Macula</th>
<th>Fd-OCT</th>
<th>ISL/OSL present</th>
<th>RPE thinned</th>
<th>Deposits above Bruch’s membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2597</td>
<td>F</td>
<td>20.6</td>
<td>0.2</td>
<td>1.2</td>
<td>RCD (20.5)</td>
<td>Abnormal</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2512</td>
<td>F</td>
<td>28.5</td>
<td>1.6</td>
<td>0</td>
<td>NR (5.7)</td>
<td>Abnormal</td>
<td>–</td>
<td>–</td>
<td>(+)</td>
<td>+</td>
</tr>
<tr>
<td>2301</td>
<td>F</td>
<td>15.8</td>
<td>0.54</td>
<td>0.23</td>
<td>NR (12.3)</td>
<td>Abnormal</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2293</td>
<td>M</td>
<td>20.8</td>
<td>0.6</td>
<td>1.7</td>
<td>RCD (20.5)</td>
<td>Abnormal</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2296</td>
<td>M</td>
<td>11.9</td>
<td>1.85</td>
<td>NT</td>
<td>NT</td>
<td>Abnormal</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2621</td>
<td>M</td>
<td>13.6</td>
<td>1.67</td>
<td>0.13</td>
<td>NR (8.1)</td>
<td>Abnormal</td>
<td>+</td>
<td>–</td>
<td>(+)</td>
<td>+</td>
</tr>
<tr>
<td>2213</td>
<td>F</td>
<td>13.5</td>
<td>1.85</td>
<td>0.01</td>
<td>RCD (11.4), NR (13.5)</td>
<td>Normal</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>2294</td>
<td>F</td>
<td>16.6</td>
<td>0.6</td>
<td>0.08</td>
<td>RCD (4.4)</td>
<td>Normal</td>
<td>–</td>
<td>–</td>
<td>(+)</td>
<td>+</td>
</tr>
</tbody>
</table>

*, siblings; VA, visual acuity (log MAR); †, nystagmus; GVF, Goldman visual field (III4e, solid angle); RCD, rod-cone dysfunction; NR, non-recordable; NT, not tested; ILM, inner limiting membrane; ONL, outer nuclear layer; ISL/OSL, inner/outer segment layer; RPE, retinal pigment epithelium; +, yes; –, no.
Patients with mutations in \textit{BBS1}

(A) Patient #2301: mutation: D8D / R483X age: 15.8 yrs

(B) Patient #2597: mutation: M390R / M390R; age: 20.6 yrs

(C) Patient #2293: mutation: M390R / N524del, age: 20.8 yrs

(D) Patient #2512: mutation: M1V / M1V, age: 28.8 yrs

Fig. 2. Horizontal Fd-OCT scans (5 mm) (left panel) and corresponding fundus photograph (right panel) of patients with mutations in \textit{BBS1}. Not identifiable ISL or OSL in the Fd-OCT scan were associated with atrophic macular changes in patient #2301 (A). Present ISL/OSL layer, ILM wrinkling and enlarged space within the ONL were visible in patient #2597 (B) and # 2293 (C) with clinically early maculopathy. Deposits above Bruch’s membrane are visible in macular scan of patient #2512 (D).
3.1. Retinal morphology

All patients with mutations in BBS1 showed some degree of maculopathy, whereas this was present in only 2 of 4 patients in the BBS10 group. Fd-OCT image acquisition was possible in all patients including those with nystagmus and fixation instability associated with low VA. Microstructural findings of retinal layers imaged are shown in Fig. 2 (BBS1) and Fig. 3 (BBS10) and summarized in Table 2.

Four cases were selected based on the genetic heterogeneity:

- **BBS1**: Case 2597, homozygous mutation in M390R (Fig. 2B). This patient with polydactyly, obesity and retinopathy was diagnosed with BBS at age 17.7 years and was re-evaluated 3 years later. Examination first showed a VA of 0.1 logMAR, slightly constricted visual field with paracentral scotoma and an early maculopathy in both eyes. Repeated ERG assessment demonstrated a progressive rod-cone dysfunction. Ophthalmic examination revealed stable VA and retinopathy at follow up visits (Table 2, Fig. 2). Fd-OCT macular scan demonstrates intact inner, but disrupted outer retinal layers. The space within the ONL or the inner and outer layers in the foveal area appears to be enlarged when compared with the control scan. Analysis of outer retinal layers was made difficult because of the disruption and possibly layer re-organization. It could not be determined whether the layer posterior to the OLM was consistent with the inner or outer segment layer because of non-visible connecting cilia. The photoreceptor inner or outer segment layer was only seen in the foveal area with gradual thinning centrifugal of the fovea. The RPE layer showed drastic thinning with a resulting higher reflectivity from the chorioid.

- **BBS1**: Case 2512, homozygous mutation in M1V (Fig. 2D). This patient with obesity, polydactyly, retinopathy and learning disability was diagnosed with BBS at age 5. Her ERG was non-recordable above noise at that time. Visual function progressed from 0.3 logMAR at age 8 to 1.6 logMAR at age 28. VF constriction was progressive from 20 degrees at age 13 to non-detectable with the III4e test target by age 28. Her most recent fundus examination showed an atrophic maculopathy, constricted vessels and general retinopathy with some minor spiculae pigmentation. Unlike the other patients studied, analysis of inner retinal layers demonstrated thinning. The outer retinal layers, including inner and outer segment layer, were not definable. The subfoveal increase in reflectivity above the RPE layer may have indicated a remaining inner or outer segment layer. The RPE was thinner than in Case 2597. Areas of increased reflectivity, almost like deposits, were seen above Bruch’s membrane.

- **BBS10**: Case 2213, heterozygous mutation in C91W and A474fs483X (Fig. 3C). This patient had clinodactyly, obesity, polycystic kidneys, learning disability and retinopathy and was diagnosed at age 8. VA dropped from 0.5 logMAR at the first visit to 1.6 logMAR at age 13.6. Nystagmus occurred from age 10 onwards. Fundus examination demonstrated a moderate maculopathy, optic atrophy and thinned retinal vessels. Retinal layer analysis demonstrated internal limiting membrane wrinkling, intact but thinned inner retinal layers and an enlarged space within the outer nuclear layer. Inner and/or outer segment layers appeared to be present within the fovea. ‘Deposits’ above Bruch’s membrane were similar to the patient described above.

4. Discussion

High-speed and high-resolution Fd-OCT allowed a detailed analysis of retinal layers in all patients with BBS tested. Unstable fixation or nystagmus did not interfere with image quality. The common characteristics of the macular scans were preserved inner retinal layers and outer nuclear layer, disrupted inner and outer segment layer and thinned RPE. Reduced, but present photoreceptor inner or outer segment layer within the foveal area was identifiable in most of the patients. A differentiation between inner and outer segment layers was not always possible due to reduced thickness and non-identifiable connecting cilia. Data from younger patients with less advanced retinopathy might yield insight into inner segment/outer segment photoreceptor layer changes including the connecting cilia.

No significant genotype-phenotype correlation was observed. To our knowledge, this is the first in vivo high-resolution retinal layer analysis in patients with mutations in BBS1 or BBS10. Preservation of inner retinal layers agrees with the recent in-vivo study by Azari et al. (2006) and with histopathological studies of a patient with BBS (Lahav et al., 1977; Runge, Calver, Marshall, & Taylor, 1986). Our observation supports a retinal electron microscopic study from an 18-year old patient demonstrating nuclei and inner segments but no outer segments within the macular area (Lahav et al., 1977). Animal models of Bbs4 mice showed a general retinopathy, which was detected earlier with histopathology than with ophthalmoscopy. ERGs in these animals revealed a cone-rod dysfunction by week 4 (Eichers et al., 2006). Histopathology
demonstrated thinned photoreceptors with loss of nuclei and shortening of inner and outer segment layer. Deposits adjacent and anterior to Bruch's membrane, which were more often evident in patients with mutations in \textit{BBS10} than with mutations in \textit{BBS1}, might be similar to the abnormal material described in patients with RP (Brosnahan, Kennedy, Converse, Lee, & Hammer, 1994; Duvall, McKechnie, Lee, Rothery, & Marshall, 1986).

Fig. 3. Horizontal Fd-OCT scans (5 mm) (left panel) and corresponding fundus photograph (right panel) of patients with mutations in \textit{BBS10}. Maculopathy is early to moderate in patient #2296 (A) and #2621 (C) but absent in the other 2 patients (B) (D). ISL/OSL are identifiable in all but patient #2213 (B). Deposits above Bruch's membrane are visible in all patients (A-D, left panel).
Those data from patients with autosomal dominant RP depict deposits of abnormal material between the RPE and inner collagenous layer of Bruch’s membrane containing PAS positive material, lipids, calcium and iron. Whether the deposits are signs of more advanced retinopathy or a faster rate of retinal layer degeneration is not known. Clinical assessment of a larger patient cohort with mutations in BBS1 and BBS10 indicate a parallel rate of progression in the two groups, but a more advanced deterioration at an earlier age in the BBS10 group (Heon, Gerth, Elia, & Munier, 2007).

Internal limiting membrane wrinkling, which is not an uncommon sign in early stages of retinal dystrophies, was identified in 3 patients. The macula in those patients showed an enlarged space within the outer nuclear layer or the inner and outer layers in the foveal area. This effect might be caused by retinal thinning and ‘collapsing’ of retinal layers.

In summary, Fd-OCT allowed excellent imaging of retinal changes seen in BBS and did not reveal any significant difference between patients carrying mutation in BBS1 or BBS10. Intraretinal ‘deposits’ seen mostly in BBS10 are possibly related to retinal re-organization. More advanced imaging techniques such as adaptive optics OCT (Zawadzki, Jones et al., 2005) could help further characterize the nature of retinal changes in advanced retinal dystrophy associated with BBS mutations. The in vivo imaging of degenerative changes related to BBS will contribute to a better understanding of the processes, which leads to photoreceptor loss. Characterization of those changes is important to best design novel therapeutic approaches.

Acknowledgments

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References


