Outer Retinal Structure in Best Vitelliform Macular Dystrophy

David B. Kay, BS; Megan E. Land, BS; Robert F. Cooper, BS; Adam M. Dubis, PhD; Pooja Godara, MD; Alfredo Dubra, PhD; Joseph Carroll, PhD; Kimberly E. Stepie, MD

IMPORTANCE Demonstrating the utility of adaptive optics scanning light ophthalmoscopy (AOSLO) to assess outer retinal structure in Best vitelliform macular dystrophy (BVMD).

OBJECTIVE To characterize outer retinal structure in BVMD using spectral-domain optical coherence tomography (SD-OCT) and AOSLO.

DESIGN, SETTING, AND PARTICIPANTS Prospective, observational case series. Four symptomatic members of a family with BVMD with known BEST1 mutation were recruited at the Advanced Ocular Imaging Program research lab at the Medical College of Wisconsin Eye Institute, Milwaukee.

INTERVENTION Thickness of 2 outer retinal layers corresponding to photoreceptor inner and outer segments was measured using SD-OCT. Photoreceptor mosaic AOSLO images within and around visible lesions were obtained, and cone density was assessed in 2 subjects.

MAIN OUTCOME AND MEASURE Photoreceptor structure.

RESULTS Each subject was at a different stage of BVMD, with photoreceptor disruption evident by AOSLO at all stages. When comparing SD-OCT and AOSLO images from the same location, AOSLO images allowed for direct assessment of photoreceptor structure. A variable degree of retained photoreceptors was seen within all lesions. The photoreceptor mosaic immediately adjacent to visible lesions appeared contiguous and was of normal density. Fine hyperreflective structures were visualized by AOSLO, and their anatomical orientation and size were consistent with Henle fibers.

CONCLUSIONS AND RELEVANCE The AOSLO findings indicate that substantial photoreceptor structure persists within active lesions, accounting for good visual acuity in these patients. Despite previous reports of diffuse photoreceptor outer segment abnormalities in BVMD, our data reveal normal photoreceptor structure in areas adjacent to clinical lesions. This study demonstrates the utility of AOSLO for understanding the spectrum of cellular changes that occur in inherited degenerations such as BVMD. Photoreceptors are often significantly affected at various stages of inherited degenerations, and these changes may not be readily apparent with current clinical imaging instrumentation.
best vitelliform macular dystrophy (BVMD), also known as vitelliform macular dystrophy type 2 or Best disease (OMIM 607854; BEST1), is an autosomal dominant form of macular degeneration of variable penetrance characterized by varying accumulation of yellowish vitelliform material in the macula.1,2 Affected individuals also show a reduction in the electrooculogram light peak but a normal full-field electroretinogram.1,3 Mutations in BEST1 on chromosome 11q13 encoding bestrophin 1 cause BVMD.4-6 Bestrophin 1 is an integral membrane protein that has been localized to the basolateral membrane of the retinal pigment epithelium (RPE) and is thought to be a calcium-sensitive chloride channel protein or influences the regulation of calcium channels.8

The clinical appearance of BVMD varies by the stage of the disease.9 Initially, retinal fundi may appear normal (previteliform). Characteristically, there is development of macular fluid- and debris-filled retinal detachments forming a yellow yolklike or vitelliform lesion or lesions. With time, the vitelliform material may become more heterogenous with varying accumulation of yellowish vitelliform material at the edges of the lesion (vitelliruptive). Eventually, localized atrophy and fibrosis develop in the location of the vitelliform lesion. Despite the presence of vitelliform lesion(s), vision is usually good in earlier stages of the disease, with visual acuity of 20/40 or better being reported in 76% of individuals younger than 40 years.9 Normal acuity can be maintained in individuals having substantial photoreceptor degeneration.10,11 Thus, the good visual acuity in patients with BVMD does not necessarily inform about the degree of photoreceptor degeneration.

Histopathologic findings from BVMD donor eyes are limited but demonstrate abnormal accumulation of lipofuscin granules in the RPE12-15 and photoreceptor degeneration over areas of intact RPE.16,17 Recently a knock-in mouse model of BVMD showed increased accumulation of lipofuscin in the RPE and deposition of subretinal debris composed of unphagocytosed photoreceptor outer segments and lipofuscin granules.18 It is hypothesized that impairment (rather than loss) of RPE to fully degrade phagocytosed outer segments leads to photoreceptor degeneration in BVMD, either alteration of the ionic milieu of the subretinal space due to bestrophin mistargeting or loss of cell-to-cell contact.13,16

Optical coherence tomography (OCT) imaging techniques allow for noninvasive assessment of retinal structure, and numerous studies have used this imaging approach to assess outer retinal structure in BVMD.19-23 Optical coherence tomography imaging has shown that the characteristic vitelliform lesions of BVMD are the result of accumulation of material in the subretinal space above the RPE and below the outer segments of the photoreceptors.20,21,24,25 Also, despite bestrophin 1 being localized to the RPE, OCT has shown significant changes to outer retinal structure evident at various stages of the disease, and it has been suggested that thickening of the reflective layer corresponding to the photoreceptors may be one of the earliest anatomical changes visible by OCT with BVMD.20,21,26 However, examples exist where the resolution of existing OCT technology is not sensitive enough to detect pronounced photoreceptor disruption.27-29 Thus, despite the OCT findings in BVMD, the nature of photoreceptor structure in BVMD remains unclear.

Adaptive optics imaging systems enable cellular-resolution imaging of the human retina, allowing for direct visualization of the cone and rod photoreceptor mosaic.30,31 To better understand photoreceptor structure across the spectrum of BVMD, we used spectral-domain OCT (SD-OCT) and adaptive optics scanning light ophthalmoscopy (AOSLO) to assess retinal structure in 4 members of the same family who are at various stages of BVMD and have a known BEST1 mutation.

Table. Patient Demographics

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>Type of Lesion</th>
<th>Visual Acuity</th>
<th>Axial Length, mm</th>
<th>Eye Imaged With AOSLO</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV-3/M/16</td>
<td>Early vitelliform</td>
<td>20/20</td>
<td>22.57</td>
<td>OS</td>
</tr>
<tr>
<td>IV-2/F/18</td>
<td>Vitelliform with early vitelliruptive</td>
<td>20/20</td>
<td>22.72</td>
<td></td>
</tr>
<tr>
<td>III-5/F/50</td>
<td>Late vitelliruptive</td>
<td>20/30</td>
<td>21.11</td>
<td>OD</td>
</tr>
<tr>
<td>III-4/F/59</td>
<td>Atrophic</td>
<td>20/200</td>
<td>23.14</td>
<td>OS</td>
</tr>
</tbody>
</table>

Abbreviation: AOSLO, adaptive optics scanning light ophthalmoscopy. * See eFigure 1 in Supplement for complete pedigree. ** Same lesion type in both eyes. *** Snellen.

Methods

This prospective study was conducted in accordance with the tenets of the Declaration of Helsinki and with institutional review board approval. Four members of a family with a previously identified mutation, p.Arg218Cys (c.652C->T) (University of California Ophthalmic Molecular Diagnostic Laboratory), in BEST1 reported to be a causative mutation in BVMD32 and with clinical findings consistent with BVMD participated (Table and eFigure 1 in Supplement). The p.Arg218Cys mutation is predicted to affect the charge of the bestrophin protein, altering its function.32 Visual acuity was assessed, and a comprehensive eye examination including fundus photography was performed for all 4 subjects. The eyes of each patient were dilated using 1 drop of phenylephrine (2.5%) prior to having microperimetry performed, after which accommodation was suspended using 1 drop of tropicamide (1%) for subsequent high-resolution imaging. Axial length was measured using an IOL Master (Carl Zeiss Meditec). Macular microperimetry was performed using the Spectral OCT/SLO MP system (OPKO Instrumentation) after a brief training to allow for familiarization of the test. A Polar 3 stan-
A standardized grid composed of 28 points arranged in 3 concentric circles (2.3°, 6.6°, and 11° in diameter from the fovea, 4 points in the innermost circle, and 12 in the middle and outer circles) was performed using a Goldman III stimulus, a 200-millisecond duration, and test strategy 4-2. Results were compared with previously published normative data.33

Volumetric images of the macula were obtained using Cirrus HD-OCT (Carl Zeiss Meditec). Volumes were nominally 6 mm × 6 mm and consisted of 128 B-scans (512 A-scans per B-scan). Retinal thickness was assessed using the built-in macular analysis software (software version 5.0), which is automatically generated by calculating the difference between the in-
ner limiting membrane and RPE boundaries. The software’s “fovea finder” algorithm was used to determine the location of the fovea on the line scanning ophthalmoscope image. Additional high-density line scans (1000 A-scans per B-scan; 100 repeated B-scans) were acquired through the foveal center in the study eye of each participant using the Bioptigen SD-OCT (Bioptigen Inc). Line scans were registered and averaged to reduce speckle noise in the image using previously described techniques and were acquired in both the horizontal and vertical direction. All scans shown in the Figures are from the Bioptigen device. Numerous naming conventions exist in the literature for the outer hyperreflective layers in SD-OCT scans, so it is important to define the one used herein. Shown in Figure 1 is a horizontal line scan from a normal control and a corresponding longitudinal reflectivity profile, showing the identity of the bands analyzed. The innermost band corresponds to the external limiting membrane, the second band corresponds to the inner segment ellipsoid, the third band corresponds to the outer segment/RPE interface (RPE1), and the fourth band corresponds to the RPE (RPE2). The peak-to-peak distance between the external limiting membrane and inner segment ellipsoid is taken as the length of the inner segments (ISs), while the peak-to-peak distance between the inner segment ellipsoid and the outer segment/RPE interface is taken as the length of the outer segments (OSs). While these may not correspond precisely to the absolute IS or OS length, we used these same definitions in an extensive previously published normative data set. We examined the IS and OS length across the horizontal line scan from each subject, sampling the scan at 0.2-mm intervals. We excluded the central BVMD-related lesion from further analysis, similar to a previous report. Images of the photoreceptor mosaic were acquired using a previously described AOSLO. Images were obtained using an Inphenix 775-nm superluminescent diode with a 12-nm full-width-at-half-maximum bandwidth. The fovea and surrounding areas affected by pathology were imaged in each patient. Parafoveal images (about 0.65° from fixation) were acquired by instructing the patient to fixate on the corners or edges of the imaging raster, while more eccentric images were acquired using an internal fixation target. Intraframe distortions within the AOSLO retinal images were corrected as previously described. Registration of frames within a given image sequence was performed using a “strip” registration method, in which the images were registered by dividing the image of interest into strips, aligning each strip to the location in the reference frame that maximizes the normalized cross-correlation between them. Once all the frames were registered, the 50 frames with the highest normalized cross-correlation to the reference frame were averaged to generate a final image with an increased signal to noise ratio.

These registered and averaged AOSLO images were then montaged using Adobe Photoshop (Adobe Systems Inc). The montage was aligned to the color fundus images and the line scanning ophthalmoscope image from the Cirrus HD-OCT, which was exported with the location of the foveal pit marked. Scaling of the images was done based on the expected scale...
of each image and alignment was done manually using blood vessel patterns. Cone density was assessed using 55 μm × 55 μm sampling areas adjacent to the visible lesion in 2 subjects and near the fovea within the active lesion in all 4 subjects using a previously described semiautomated algorithm.40 The distance between the sampled area and the foveal pit was measured, enabling comparison of density values with previously published normative values.

Results

Four affected subjects from a family with BVMD with known p.Arg218Cys mutation in BEST1 participated (eFigure 1 in Supplement). All family members were found to be at different stages of the disease, as summarized in the Table. The SD-OCT and AOSLO imaging findings were unique to each stage (Figures 2, 3, 4, and 5). Macular microperimetry performed within a 6° radius of the fovea revealed areas of subnormal individual point sensitivities in regions corresponding to clinical visible retinal lesions (Figures 2C, 3C, 4C, and 5C) in all but patient IV-3 with early vitelliform findings. In patient IV-2, decreased point sensitivities were seen both in regions immediately surrounding the vitelliform lesion and overlying the lesion itself.

Measurement of IS and OS layer thickness was performed using the SD-OCT horizontal line scan in all 4 subjects. Shown in Figure 6 are the IS and OS thickness profiles in areas immediately adjacent to clinical visible lesions for all 4 subjects compared with data from a previously published normative group.41 Thickness values were not calculated over the clinically visible lesion. All 4 subjects were found to have IS and OS thickness values within 2 SDs of the normative mean.

We sought to further assess photoreceptor structure in the retinal area adjacent to the BVMD lesions. In 2 patients having lesions with a clear boundary, we were able to obtain AOSLO montages that were large enough to encompass the entire lesion (Figure 2 and eFigure 2 in Supplement, full montage of clinical vitelliform lesion, patient IV-2). We assessed cone density just nasal to the lesion boundary in both patients IV-3 and IV-2 and determined that the areas sampled were 1° from the foveal center. The cone mosaic appeared contiguous and cone density was 55 900 cones/mm² in patient IV-3 and 43 700 cones/mm² in patient IV-2. Both values are within the normal range for this retinal eccentricity.41

In the SD-OCT scans of 1 of the subjects (patient IV-2), we noticed significant hyperreflective material in the outer nuclear layer. This has been previously reported in BVMD42 and is attributed to the physical deformation of the Henle fiber layer by the underlying vitelliform lesion. Inspection of the SD-OCT volume revealed the strongest signal in the inferior retina, just nasal to the fovea. The AOSLO images from this same location focused in the inner retina revealed thin hyperreflective structures running perpendicular to the nerve fiber layer (Figure 7). The anatomical location and orientation are consistent with that of Henle fibers, and the diameter of these
structures (mean = 2.76 [0.32] μm) is consistent with previous histology reports.43

Discussion

In our study, we used SD-OCT and AOSLO to assess outer retinal structure in 4 members of a single family harboring a previously reported BEST1 mutation (p.Arg218Cys). The phenotypes ranged from early vitelliform changes to a central atrophic area. Disruption of the cone mosaic was evident in the AOSLO images at all stages of BVMD presented herein, including the patient with the earliest stage of vitelliform clinical findings (Figure 2), suggesting this is an early finding in patients with BVMD. The degree of this photoreceptor disruption varied by stage of disease and was often patchy with areas of significant photoreceptor disruption surrounded by areas of a contiguous photoreceptor mosaic, even in the patient with advanced atrophy and fibrosis (Figure 5). Disruption of visualization of cone structure on AOSLO does not necessarily mean the cone cell has been lost. When comparing SD-OCT and AOSLO images from the same location, the AOSLO images allowed for better understanding of the degree of retained photoreceptor structure at that location. This is illustrated in the patient with late vitelliruptive changes (Figure 4). The SD-OCT of this individual shows significant disruption of the hyperreflective inner segment ellipsoid band in the areas of subretinal nodules, but the AOSLO images reveal islands of contiguous cone mosaic adjacent to areas of significant disruption.

Previous studies have suggested that loss of photoreceptors in BVMD could be widespread and not necessarily confined to the clinically apparent lesions, and support for this comes from the fact that bestrophin, the RPE membrane protein encoded by BEST1, is found throughout the retina in individuals unaffected by BVMD.17 Kay et al21 recently showed increased photoreceptor thickness on SD-OCT in patients with BVMD when compared with normal controls within the macular region. Based on their findings, they conclude that the primary anatomical impact is at the photoreceptor level. Certainly, our finding that the photoreceptor mosaic is disrupted in the earliest stage of clinical vitelliform findings would be consistent with this proposed etiology, but our finding of normal IS and OS thickness and normal cone density in retinal areas adjacent to visible lesions argues against a diffuse structural deficit in BVMD. One possible explanation is that the authors of the previous study did not correct the lateral scale of their SD-OCT scans for individual differences in axial length, meaning that different extents of retina contributed to the analysis in each retina. Moreover, since the previous analysis averaged the thickness measurements across the scan, it is unclear if the retina was indeed uniformly affected or if a small retinal area was severely abnormal.21 Nevertheless, while our findings do not support diffuse disruption of the cone mosaic...
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tioningnormally.

Interestingly, macular microperimetry revealed areas of
subnormal point sensitivities in areas surrounding the vitel-
liform lesion in patient IV-2. Both SD-OCT and AOSLO showed
normal outer retinal anatomy within these regions. These re-
duced point sensitivities may be the result of eye movements
reducing the specificity of registration to the fundus. How-
ever, it may also be possible that functional loss of vision
precedes anatomical outer retinal structural loss. High-
resolution microperimetric assessment using adaptive optics
technology has been described.44,45 We also observed this effect in 1 of our
subjects (patient IV-2); however, we also observed the pres-
ence of fine hyperreflective structures running perpendicular to nerve
fiber bundles, consistent with known anatomy of Henle fibers.

example, presence of a vitelliform lesion, large drusen, or pig-
ment epithelial detachment alters the orientation of the fi-
bers of Henle as they traverse the lesion, altering their reflec-
tivity on SD-OCT.46,47 We also observed this effect in 1 of our
subjects (patient IV-2); however, we also observed the pres-
ence of fine hyperreflective structures running perpendicular to the nerve fiber bundles in the AOSLO images at the same retinal
location (Figure 7). Their anatomical location, orienta-
tion, and size are consistent with that of Henle fibers. As seen
with SD-OCT, this demonstrates that when imaged with AO-
SLO, outer retinal disruptions can alter the appearance of the inner retina, and this should be taken into consideration when
analyzing such images.

A potential limitation of the current study is that all 4
subjects have the same genetic mutation in BEST1. While our
data reveal a spectrum of clinical and subclinical findings
associated with this particular mutation, it is not possible to
extend our findings on the integrity of the cone mosaic to
other mutations. Future investigations should include high-
resolution imaging of other individuals with different muta-
tions in BEST1 to investigate possible genotype-dependent
differences in photoreceptor structure.

In summary, we provide evidence from cellular imaging
with AOSLO that photoreceptor structure can be retained
within active BVMD lesions, even in apparently atrophic
lesions. This photoreceptor structure is capable of support-
ing rather good visual acuity, because visual acuity in the
eyes imaged herein ranged from 20/20 to 20/50. In addition,
our SD-OCT and AOSLO data show normal photoreceptor

Figure 6. Assessment of Photoreceptor Layer Thickness in Best
Vitelliform Macular Dystrophy

A, Plot of inner segment (IS) length as a function of retinal location along the
horizontal meridian. B, Outer segment (OS) length as a function of retinal
location along the horizontal meridian. The black line indicates normative data
from 93 people, mean (SD) age, 25.7 (8.2) years. The shaded gray area is ±2 SD.
Thickness values were not calculated over visible lesion(s).

Figure 7. Visualization of the Henle Fiber Layer in Best Vitelliform
Macular Dystrophy

A, Presence of the vitelliform lesion has altered the angle of the retina structure,
allowing for visualization of the Henle fiber layer on spectral-domain optical
coherence tomography (arrows). B, Adaptive optics scanning light
ophthalmoscopy imaging at this same location focused at the level of the inner
retina reveals thin hyperreflective structures running perpendicular to nerve
fiber bundles, consistent with known anatomy of Henle fibers.

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structure in retinal areas outside the clinically visible lesion, in contrast to previous reports\(^4\) but consistent with previous findings with AOSLO.\(^4\) This may represent a specific feature of the mutation studied herein or be due to different imaging and measurement procedures. Regardless, our study highlights the utility of AOSLO imaging in directly delineating the degree of retained photoreceptor structure in diseases like BVMD. In particular, combining information from SD-OCT with that from AOSLO gives a complementary view of outer retinal structure and provides a more sensitive approach for measuring photoreceptor structure than either alone.

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**REFERENCES**


