Pathologic Changes of Cone Photoreceptors in Eyes With Occult Macular Dystrophy

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PURPOSE. Occult macular dystrophy (OMD) is an inherited retinal disease characterized by a progressive decrease of vision and appearance of normal fundus. To determine the pathologic features of OMD, we investigated the alternation of the photoreceptors using quantitative image analysis.

METHODS. We studied 22 eyes of 11 OMD patients. Three of them had a mutation (R45W) in RP1L1. The relative intensities of the ellipsoid zone in the spectral-domain optical coherence tomography (SD-OCT) images and the density of the cone photoreceptors in the adaptive optics (AO) fundus images of the OMD patients were compared to those of normal controls.

RESULTS. The relative intensities of the ellipsoid zone in the SD-OCT images of patients with OMD were significantly lower (P < 0.001) by an average of 16% compared to that of the normal controls. Normal cone mosaics were not observed in the AO images of the macula in the eyes with OMD. The mean ± SD of cone density of the 9 OMD patients was 1970 ± 884 cells/mm² at 2°, 1124 ± 483 cells/mm² at 3°, and 1288 ± 715 cells/mm² at 4° nasal to the fovea. The cone densities at 2°, 3°, and 4° nasal to the fovea of OMD were significantly lower than those of the normal controls (P < 0.001).

CONCLUSIONS. A sparse array of cone photoreceptors with significantly reduced density of the macula is one of the morphologic features of OMD.

Keywords: occult macular dystrophy (OMD), adaptive optics (AO), spectral-domain optical coherence tomography (SD-OCT), RP1L1

Occult macular dystrophy (OMD) is a hereditary retinal disease that was first described by Miyake et al.1,2 They reported that OMD is a unique form of macular dystrophy characterized by normal fundus appearance, normal fluorescein angiogram (FA), and normal full-field electroretinogram (ERG) despite a slow progressive decrease of the patient’s visual acuity.1–3 Therefore, the diagnosis of OMD is based on abnormal focal macular ERGs and/or multifocal ERGs (mfERGs).

Akahori et al.4 reported that dominant mutations in the retinitis pigmentosa 1-like 1 (RP1L1) gene cause OMD. Several other groups also have reported that mutations of the RP1L1 cause OMD5–8; however, only approximately half of OMD patients have causative mutations in the RP1L1.6 Thus, OMD is considered to be a set of genetically heterogeneous disease with similar retinal dysfunctions, although the exact morphologic features still are debatable.

Although the fundi of eyes with OMD are essentially normal, morphologic abnormalities in optical coherence tomography (OCT) images have been reported.9 Recent advancements of spectral-domain OCT (SD-OCT) have allowed investigators to detect subtle morphologic alterations of the photoreceptor layer.10–13 At present, the subtle differences in the OCT images are used for the diagnosis of OMD. The photoreceptor abnormalities detected in SD-OCT images have been suggested to be the main pathologic alterations in eyes with OMD.

Adaptive optics (AO) retinal imaging is another method of analyzing the photoreceptors in situ. Kitaguchi et al.14 examined AO fundus images and found an absence of normal cone photoreceptor mosaics in eyes with OMD. The AO retinal images also showed lower cone densities in the macula of two OMD patients compared to those of controls.15,16

The purpose of this study was to determine the pathologic features of OMD. To accomplish this, we performed quantitative analysis of the photoreceptor layer on the SD-OCT images from 11 OMD patients. In addition, we used the flood-illuminated AO fundus camera images to assess the density of the cone photoreceptors.

METHODS

This was an observational case series study. The procedures used were approved by the Institutional Review Board of Nagoya University Graduate School of Medicine (1067-2). Signed informed consent was obtained from all patients, and all of the procedures conformed to the tenets of the Declaration of Helsinki.

We reviewed the medical records of 11 patients who had been diagnosed with OMD and had undergone medical examinations between August 2011 and September 2013 at the Nagoya University Hospital. The diagnosis of OMD was...
made through a history of progressive decrease of the visual acuity in eyes with normal ophthalmoscopic appearance, normal FAs, normal full-field ERGs, and reduced focal macular ERGs (ER-80; Kowa, Nagoya, Japan) and/or mfERGs (VERIS; EDI, San Mateo, CA, USA). In addition, we confirmed that the fundus autofluorescence (FAF) images had no obvious abnormalities in the nine patients who underwent this examination.

Patients underwent a comprehensive ocular examination, including measurements of the best-corrected visual acuity (BCVA), fundus examination, visual field tests by a Humphrey Field Analyzer (Carl Zeiss Meditec, Dublin, CA, USA), FAF (Spectralis; Heidelberg Engineering, Heidelberg, Germany), SD-OCT (Spectralis; Heidelberg Engineering), and flood-illuminated AO fundus camera photography (rtx1; Imagine Eyes, Orsay, France).

**Analysis of the Ellipsoid Zone in SD-OCT Images**

Spectral-domain OCT was used to record horizontal cross-sectional images of the fovea. Scans (30° wide) through the center of the fovea were obtained by averaging 100 frames with an eye-tracking system. Optical coherence tomography images were obtained from both eyes of the 11 patients (n = 22) and 40 eyes of 40 age- and axial length-matched control subjects (n = 40). In the control subjects, the better image was selected when the images of both eyes were suitable.

To quantify the integrity of the ellipsoid zone in the SD-OCT images, we measured the relative intensity of the ellipsoid zone relative to other parts of the retina in the horizontal SD-OCT images using the ImageJ software (version 1.48; http://imagej.nih.gov/ij/; provided in the public domain by the National Institutes of Health, Bethesda, MD, USA). We used the method reported by Hood et al.17 with modifications. The ellipsoid zone, RPE/Bruch’s membrane complex/choroid border, and the nerve fiber layer (NFL)/ganglion cell layer (GCL) border were manually segmented. In Figure 1, the ellipsoid zone has been cropped to 5 pixels in depth (3.87 μm/pixel) and 6 pixels in width (12.0 ± 0.65 μm/pixel; mean ± SD) at each location in the yellow square in an SD-OCT image of a normal control eye.

We measured the intensity of the ellipsoid zone at 11 fixed 5 × 6 pixel areas in 1° steps between ±5° from the fovea. The background of the ellipsoid zone was defined as the area between the RPE/Bruch’s membrane complex/choroid border and the NFL/GCL border extending for ±20 pixels in width. An example of the ellipsoid zone (the yellow square) and the background area (outlined in the green line) are shown in the SD-OCT image of a normal control in Figure 1A.

**Figure 1.** Spectral domain OCT images of patients with OMD. (A) Horizontal SD-OCT scans of a control eye and three eyes of OMD patients. One of the measured areas of the ellipsoid zone (yellow square) and its background (green line) are shown in the SD-OCT image of a normal control retina. The relative intensity of the ellipsoid zone is shown in the open circle below each SD-OCT image. The mean ± 2 SDs limits of the 40 normal control eyes are shown in the gray solid line and gray dotted lines, respectively. Plus degrees indicate temporal side and minus indicate nasal side. (B) The mean ± 2 SDs limits of the relative intensities of the ellipsoid zone of control subjects (n = 40, black) and the mean of those of OMD patients (n = 22, red).
AO Fundus Image Processing and Analyses

En face images within 6° of the fovea were obtained using a flood-illuminated AO fundus camera (rtx1; Imagine Eyes). The rtx1 has a resolution of 250 line pairs/mm, with a 4° × 4° imaging field and an illumination wavelength of 850 nm. The pupils were dilated using a combination of phenylephrine hydrochloride (0.5%) and tropicamide (0.5%) before imaging. Each series of 40 images acquired by the AO camera was processed to produce a final image using software programs provided by the system manufacturer (CK v0.1 and AOdetect v0.1; Imagine Eyes). The raw images that had artifacts due to eye blinking and saccades were automatically eliminated before the averaging was performed. Montage images were created using i2k Retina software (DualAlign LLC, Clifton Park, NY, USA).

The cell densities of the left eye of the nine OMD patients (n = 9) and 20 eyes of 20 age- and axial length–matched normal controls (n = 20) were measured at 2°, 3°, and 4° from the fovea on the nasal side. For the control subjects, the eye with the better image was selected when images of both eyes were suitable. The area of analysis was 200 × 200 pixels, which corresponds to approximately 160 μm². The cell density was automatically calculated by the software, which subsequently was corrected manually. To determine the degree of agreement of the manual correction among the graders, the cones were counted by three investigators (AN, NY, USA).

The cell densities of the left eye of the nine OMD patients were compared to those of 20 normal eyes. We did not assess the cone density within 1° from the foveal center because the cone density was too high and the cone diameter was too small within this area in the normal eyes and, thus, exceeded the resolution limits of the camera.

To investigate the characteristics of the photoreceptor cells, we compared the reflectance profiles of the cells using a method similar to those in previous reports. Single raw AO images at 2° and 4° nasal to the fovea were obtained from the left eye of the nine OMD (n = 9) and 20 eyes of 20 age- and axial length–matched normal controls (n = 20). We obtained the reflectance profiles of 20 pseudorandomly selected cells from each single raw AO image. To compare the extent of the reflectance of the cells, the profiles were fit to a Gaussian curve and the full width at half maximum (FWHM) was calculated using ImageJ. The FWHM was compared between the OMD patients and normal controls. Examples of the reflectance profiles of the cells between the OMD patients and normal controls. The FWHM was calculated from the Gaussian curve fit from the reflectance profile of a single cell. The FWHM was calculated from the Gaussian function.

DNA Analyses

DNA analyses were performed as described in detail. Mutation analyses were performed on 7 of the 11 OMD patients, and the 4 other patients did not agree to undergo the genetic analysis. Genomic DNA was isolated from peripheral white blood cells of 7 patients. The DNA was used as the template to amplify the coding regions and flanking introns of the RP1L1 gene by PCR using primers. The PCR products were purified and used as templates for sequencing, and both strands were sequenced on an automated sequencer.

Statistical Analyses

Mann-Whitney U tests were used to determine the significance of any differences in the characteristics, relative intensities of the ellipsoid zone, cone densities, and reflectance profile of the cells between the OMD patients and normal controls. The intraclass correlation coefficient (ICC) was used to assess the consistency of measurements among the three graders.

RESULTS

The demographic, clinical, and genetic characteristics of the 11 OMD patients (seven men and four women) are shown in Table 1. The mean age was 50 ± 14 years, with a range of 19 to 69 years. The mean duration of the reduced visual acuity was 13 ± 8 years, with a range of 2 to 25 years. Both eyes were affected in all patients. The mean BCVA was 20/60 with a range from 20/200 to 20/40. Three of the 11 OMD patients had more than a 0.4 logMAR unit decrease in the BCVA within the 2-year follow-up period. Slit-lamp and fundus examinations revealed no remarkable findings. One patient (Case 7) had an autosomal dominant inheritance pedigree, and the other 10 patients were sporadic cases. None of the patients was related (see Supplementary Figure S1 for the pedigrees of the OMD patients).

RP1L1 Mutation Analyses

An Arg45Trp mutation was found in the RP1L1 in three of the patients (Cases 1–3), and the other four patients (Cases 6 and 9–11) had no reported causative mutations in the RP1L1 (Table 1). This incidence is comparable to that from a previous report. We performed exome analysis for these four patients but did not detect any other reported causative mutations.
Arg45Trp has been identified as a dominant mutation in a Japanese OMD family, but none of the three patients had distinctive family histories as shown in Supplementary Figure S1.

Quantitative Analysis of the Ellipsoid Zone in the SD-OCT Images

The demographic characteristics of the OMD patients and normal controls are summarized in Table 2. The horizontal SD-OCT images and the relative intensities of the ellipsoid zone at ±5° from the fovea of a normal control and three representative OMD patients are shown in Figure 1A. Compared to the normal control eyes, the structure of the outer retinal layers of the eyes of OMD patients was abnormal, including the mottled appearance of the ellipsoid zone and the loss of the interdigitation zone around the macula, as has been reported previously. The ellipsoid zone at the foveola was not detected in half of the patients (Cases 1–3, 6, 7, and 11). In Figure 1A, the profile of the relative intensity of the ellipsoid zone of each subject is shown in the open circle, and the mean (±SD) limits of the 40 normal control eyes are shown by the gray solid and gray dotted lines, respectively. The mean of the relative intensity of the ellipsoid zone of the OMD patients was lower by an average of 16% compared to that of the normal controls (Fig. 1B). There were significant differences between the mean relative intensity of the ellipsoid zone of the OMD patients and that of the normal controls in all measured areas (Mann-Whitney U test; P < 0.001).

AO Image Findings

The integrity of the cone photoreceptors in the AO images of 20 normal control eyes was compared to that of nine of the 11 OMD patients. Two OMD patients (Cases 10 and 11) were excluded from the analyses due to the low quality of the images. We also excluded the data at 2° nasal to the fovea of Case 7 because only a few cones were observed, and we believed that the cell counts in this area were not reliable. A representative AO montage image of the fovea of an OMD patient (Case 3) is shown in Figure 3A. This patient did not have a normal cone photoreceptor mosaic pattern, and the cells were spread sparsely throughout this region. The RPE cells were observed clearly in the AO image of Case 6 where the ellipsoid zone was totally disrupted in the SD-OCT images (data not shown). Magnified AO images taken at 3° nasal from the fovea of a normal control eye and four representative OMD eyes are shown in Figure 3B. A well-defined cone photoreceptor mosaic pattern was observed in the normal control eyes but not in the OMD eyes. However, scattered cells were visible in these OMD patients as in Case 3. Similar findings were confirmed in the other four OMD patients whose AO images were examined.

Cell Density

The mean ± SD of the cell density of the nine OMD eyes was 1970 ± 884 cells/mm² at 2°, 1124 ± 483 cells/mm² at 3°, and 1288 ± 715 cells/mm² at 4° from the fovea on the nasal side. The mean ± SD of the cone density of the 20 normal controls was 22,981 ± 4720 cells/mm² at 2°, 18,186 ± 3032 cells/mm² at 3°, and 15,699 ± 1578 cells/mm² at 4° from the fovea on the nasal side. The mean cell densities in the OMD patients were markedly lower than those in the normal controls at all of the evaluated sites (Mann-Whitney U test; P < 0.001), although there were differences among the patients (Tables 3, 4). Case 4 had approximately the same cell density at all the measured areas, and some of the

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**Table 1.** Demographics and Clinical Characteristics of Patients

<table>
<thead>
<tr>
<th>Case</th>
<th>Age, y</th>
<th>Sex</th>
<th>RP111 Mutation</th>
<th>Inheritance</th>
<th>Symptom Duration, y</th>
<th>OD</th>
<th>OS</th>
<th>BCVA, Spherical Equivalent, D</th>
<th>Axial Length, mm</th>
<th>Estimated Distance to Angle, μm/deg</th>
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<tr>
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<td>F</td>
<td>p.Arg45Trp</td>
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<td>20/60</td>
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<td>20/160</td>
<td>20/125</td>
<td>7.25</td>
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<tr>
<td>3</td>
<td>44</td>
<td>F</td>
<td>p.Arg45Trp</td>
<td>Sporadic</td>
<td>7</td>
<td>20/200</td>
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<td>Sporadic</td>
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<td>20/50</td>
<td>20/50</td>
<td>7.25</td>
<td>20/50</td>
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<tr>
<td>7</td>
<td>58</td>
<td>M</td>
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<td>AD</td>
<td>25</td>
<td>20/50</td>
<td>20/50</td>
<td>2.25</td>
<td>20/50</td>
<td>20/50</td>
</tr>
<tr>
<td>8</td>
<td>19</td>
<td>F</td>
<td>N/A</td>
<td>Sporadic</td>
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<td>20/60</td>
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<td>20/160</td>
<td>2.50</td>
<td>20/160</td>
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</tr>
</tbody>
</table>

F, female; M, male; N/A, not available; N/D, not detected; AD, autosomal dominant; D, diopters; symptoms duration, duration since the patients first noticed a decrease in visual acuity by examination.

**Table 2.** Summary of OMD Patients’ and Normal Controls’ Characteristics of the SD-OCT Study

<table>
<thead>
<tr>
<th></th>
<th>OMD, n = 22</th>
<th>Normal, n = 40</th>
<th>P Value</th>
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<td>Age, mean ± SD, y</td>
<td>50 ± 14</td>
<td>49 ± 11</td>
<td>0.867</td>
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<td>BCVA, mean ± SD, logMAR</td>
<td>0.57 ± 0.2</td>
<td>0.0 ± 0.0</td>
<td>&lt;0.001</td>
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<td>Spherical equivalent, mean ± SD, D</td>
<td>−4.15 ± 3.6</td>
<td>−4.14 ± 3.5</td>
<td>0.963</td>
</tr>
<tr>
<td>Axial length, mean ± SD, mm</td>
<td>25.60 ± 1.8</td>
<td>25.47 ± 1.7</td>
<td>0.710</td>
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</table>
patients had a higher cell density at 4° than at 2° (Cases 6 and 7), while others had a higher density at 2° than 4° (Cases 1–3, 5, and 9).

We evaluated the degree of agreement in identifying the cones among the graders using ICCs. The ICCs (95% confidence interval) of the normal controls and OMD patients were 0.99 (0.993–0.998) and 0.89 (0.750–0.947), respectively.

Reflective Profiles of Cells

The means ± SDs of the FWHM of the reflective profiles of the cells of the nine OMD patients were 5.10 ± 0.58 μm at 2° and 4.91 ± 0.44 μm at 4°, and those of the 20 healthy controls were 3.73 ± 0.28 μm at 2° and 3.87 ± 0.24 μm at 4° (Fig. 4). The means of the FWHM in the OMD patients were 1.36 and 1.27 times larger than those of the normal controls at 2° and 4°, respectively. There were significant differences between the controls and OMD patients at both sites (Mann-Whitney U test; \( P < 0.001 \)).

**DISCUSSION**

Occult macular dystrophy is a unique macular disease that does not cause visible ophthalmoscopic abnormalities. Originally, OMD was diagnosed based on abnormal macular function as determined only by reduced focal macular ERGs or mfERGs; however, with the advancement of OCT technology, abnormal alterations of the photoreceptor layer have been detected in the eyes of OMD patients.

The SD-OCT images of the eyes of the OMD patients showed a loss of the interdigitation zone and a mottled appearance of the ellipsoid zone around the macula. We analyzed the ellipsoid zones in the SD-OCT images and found a reduction in the intensities of the ellipsoid zone in the eyes with OMD relative to those of the normal controls. Ziccardi et al. performed texture analyses of the ellipsoid zone in an OMD patient and reported a disruption of the ellipsoid zone in the macula. These findings indicate degeneration of the inner and outer segments of the photoreceptors.

In the eyes with OMD, the AO fundus images within 6° of the fovea showed an absence of normal cone photoreceptor mosaics, which is in agreement with earlier studies. The cone densities of the normal controls decreased with increasing distance from the fovea as reported in previous studies.

**TABLE 3. Cell Density of the Left Eye of Nine OMD Patients**

<table>
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<td>9</td>
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<td>1188</td>
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</table>

Locations are all nasal eccentricity, relative to fixation (cells /mm²).

**FIGURE 3.** Adaptive optics (AO) fundus images of patients with OMD. (A) Fundus image and AO images of the left eye of a patient with OMD (Case 3). Black square on fundus color image indicates the registration of the AO image montage. A magnified image of the white square on AO image is shown in (B). Yellow asterisk indicates the fovea. Scale bar: 250 μm. (B) Adaptive optics images of a normal control and 4 OMD patients taken at 3° retinal eccentricities nasal to the fovea. The cone mosaic pattern is clearly visible in normal control image but not in the OMD eyes. Scattered cells are seen in all OMD patients although the densities are different. Scale bar: 50 μm.

**FIGURE 4.** The means of the FWHM of the reflectance profiles of the cells in normal controls (n = 20, white) and OMD patients (n = 18, black) at 2° and 4° retinal eccentricities, nasal to the fovea. Error bars: standard deviation. **\( P < 0.001 \).
studies. However, the relationship between the cone density and retinal eccentricity varied among the OMD patients.

The decreased cone densities in the eyes of OMD cases were observed in AO images as in two previous reports. One of these studies reported a cone density of approximately 8000 to 9000 cells/mm² in a case with an RP1L1 mutation, while the other reported a cone density of 1193 to 6360 cells/mm² in a case without an RP1L1 mutation at similar retinal eccentricities as in our study. We found some differences in the cone densities among the studies. These differences might be explained by a difference in the stage of the disease progression. Another reason that is more likely is that it was due to differences in the cone-counting procedures used in the different studies. The reliability of the image processing of the software for automated identification of the cone photoreceptors in normal retinas has been proven but not in degenerated retinas. The filtering algorithms may not be optimal for the eyes with OMD that showed extremely low cone density, because the noise from the increased intercone space might count toward the cone signal. Thus, we believed it was a better method to perform manual corrections after the automated counting performed by the software. The intergrader agreement in our study was consistent, but was relatively lower for the OMD patients than for the normal controls.

We investigated the characteristics of the cells to confirm that the observed cell signals in the eyes with OMD were derived from cone photoreceptors, because the densities were much lower, and the appearances of the cone mosaic in the OMD patients differed from those in the normal controls. We analyzed the reflectance profiles of the cells using a method reported in previous studies. The FWHM of the reflectance profiles of the cells in the eyes with OMD was only slightly larger than that in the eyes of the normal controls. Thus, the cell signals from the patients with OMD were most likely cones.

Although the relationship between the FWHM of the reflectance profiles of the cells and the actual diameter of the cells was not clear, the larger FWHMs of OMD patients than those of normal controls may represent the enlargement of the cone cells. Wolfing et al. also reported larger diameters of the cones in eyes with cone rod dystrophy that had lower cone densities in the central macular region. One possible explanation for the enlargement of the cone cells could be that the increased intercone spaces allow the residual cones to enlarge. Another possibility for the larger diameter of the cone cells in eyes with OMD compared to those in control eyes would be that they swelled up during the course of degeneration.

The appearance of the AO images in the macula of eyes with OMD was similar to that reported for oligocone trichromacy. Oligocone trichromacy is a retinal disorder in which eyes have a reduced number of functioning cones. The AO retinal images of oligocone trichromacy patients show reduced cone density and sparse mosaics of cones remaining at the fovea. This result supports the assumption that the detected cells in this study were cones.

There are some limitations in the methods used in this study. One limitation is that the measurements of the relative intensities of the ellipsoid zone were affected by the background intensity and might not accurately reflect the differences in the intensity of the ellipsoid zone. However, we believed that it was better to adjust the intensity of the ellipsoid zone to the local background to reduce the variations in the intensities among the scans in the same eye. We adopted the method reported by Hood et al., because their method was used to assess eyes with retinal diseases with diminished cone function that appeared to be similar to the condition of the macula in OMD eyes.

Another limitation is that the FWHMs of the reflectance profiles of the cells were not the actual diameter of the cells and also different from those calculated from the voronoi cells of the AO images or the histologic data. Lastly, another limitation is that the identification of cone photoreceptors in the degenerating retina by rtx1 and the software remains to be established. The disarrayed cones that lose the waveguide feature might not be detected, and/or signals originating from other retinal structures might be detected incorrectly as the cone signals. Recent advancements in AO technology, for example, split-detector AO scanning laser ophthalmoscopy, can image the inner segments of photoreceptors and create dark-field images of the RPE, and may be able to uncover more details of the pathophysiology of eyes with OMD.

In conclusion, we assessed 11 OMD patients and compared their photoreceptors in SD-OCT images and AO images to those of normal controls. We found a sparse array of cone photoreceptors with significantly reduced density in the macula of OMD eyes compared to that in control eyes regardless of the presence or absence of an RP1L1 mutation. This supported the idea that OMD is a set of heterogeneous disease but has similar morphologic conditions. Our results showed that SD-OCT and AO imaging are effective tools for diagnosing and analyzing eyes with OMD.

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