High-Resolution Adaptive Optics Retinal Imaging of Cellular Structure in Choroideremia

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PURPOSE. We characterized retinal structure in patients and carriers of choroideremia using adaptive optics and other high resolution modalities.

METHODS. A total of 57 patients and 18 carriers of choroideremia were imaged using adaptive optics scanning light ophthalmoscopy (AOSLO), optical coherence tomography (OCT), autofluorescence (AF), and scanning light ophthalmoscopy (SLO). Cone density was measured in 59 eyes of 34 patients where the full cone mosaic was observed.

RESULTS. The SLO imaging revealed scalloped edges of RPE atrophy and large choroidal vessels. The AF imaging showed hypo-AF in areas of degeneration, while central AF remained present. OCT images showed outer retinal tubulations and thinned RPE/interdigitation layers. The AOSLO imaging revealed the cone mosaic in central relatively intact retina, and cone density was either reduced or normal at 0.5 mm eccentricity. The border of RPE atrophy showed abrupt loss of the cone mosaic at the same location. The AF imaging in comparison with AOSLO showed RPE health may be compromised before cone degeneration. Other disease features, including visualization of choroidal vessels, hyper-reflective clumps of cones, and unique retinal findings, were tabulated to show the frequency of occurrence and model disease progression.

CONCLUSIONS. The data support the RPE being one primary site of degeneration in patients with choroideremia. Photoreceptors also may degenerate independently. High resolution imaging, particularly AOSLO in combination with OCT, allows single cell analysis of disease in choroideremia. These modalities promise to be useful in monitoring disease progression, and in documenting the efficacy of gene and cell-based therapies for choroideremia and other diseases as these therapies emerge. (ClinicalTrials.gov number, NCT01866371.)

Keywords: choroideremia, adaptive optics, photoreceptors, retinal pigment epithelium

Choroideremia is an X-linked inherited retinal degeneration caused by mutations in the *CHM* gene, which encodes rabsort protein 1 (REP1). Choroideremia is a slowly progressive disease; patients initially present with nyctalopia, and progress to tunnel vision and ultimately blindness.^{1,2} However, many choroideremia patients can maintain significant central vision into their 40s and 50s.³ Retinal imaging has revealed early pigmentary changes in the periphery, followed by progressive enlargement of peripheral atrophy toward the central retina, and finally atrophy of the macula in late stages of the disease. The borders of atrophic retina exhibit characteristic scalloped edges.¹ Female carriers of choroideremia generally are asymptomatic, though patchy pigmentary changes are observed with funduscopy. This patchy fundus appearance is explained by lyonization where, rarely, a female carrier may exhibit significant vision loss as a result of skewed X-inactivation.^{4,5}

Despite genetic characterization of *CHM*, the exact mechanism of vision loss associated with choroideremia remains unknown. In general, choroideremia leads to the degeneration of the outer retina, the RPE, and the choroid/choriocapillaris.² There are several theories regarding which cell layers are primarily affected. Prevailing theories include: primary loss of the photoreceptor layer,⁶ in particular the rod photoreceptors,⁷

followed by degeneration of the RPE and choroid; primary loss of the RPE followed by degeneration of the photoreceptor layer and choroid^{8–10}; and independent loss of the photoreceptor layer and RPE followed by degeneration of the choroid.^{1,11} It is important to fully characterize the structural phenotype and progression of choroideremia with respect to these cell layers, especially in light of emerging therapies for this disease¹² and the hypothesis that, for retinal gene therapy to be functionally successful, retinal structure must remain intact.

The goal of the present study is to provide structural characterization of the cell layers involved in choroideremia through high-resolution retinal imaging. Retinal imaging comprises many distinct yet complimentary modalities, including scanning laser ophthalmoscopy (SLO), spectral domain optical coherence tomography (OCT), autofluorescence (AF), and adaptive optics scanning light ophthalmoscopy (AOSLO).¹³ The AO imaging uses a wavefront sensor and a wavefront corrector to compensate for the eye's optical aberrations and, thus, provides diffraction limited imaging through the natural optics of the eye.¹⁴ This technique has allowed noninvasive observation of the individual photoreceptors in patients with retinal disease.^{15–22} Combined, data from the various high-resolution imaging modalities can provide a thorough assess-

TABLE 1. Choroideremia Patient Data

Patient ID	Age	Axial Length, mm		Visual Acuity		Cone Density Measured*		Hyper-Reflective Spots†		Inner Retinal Microcysts Structures†		Bubble-Like Structures†	
		OD	OS	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS
13044	7	22.09	22.08	20/25	20/20	4 (3/0/1)	4 (3/1/0)	No	No	No	No	No	No
13031	9	22.4	22.46	20/20	20/20	1 (1/0/0)	0	Yes	Yes	No	No	No	No
13092	14	24.01	23.7	20/26	20/22.5	5 (5/0/0)	7 (5/0/2)	Yes	Yes	No	Yes	No	No
13099	15	23.42	23.39	−9.27dB‡	−11.8dB‡	9 (8/1/0)	9 (6/1/2)	No	No	No	No	No	No
13035	16	23.7	23.64	20/30	20/50	3 (3/0/0)	4 (4/0/0)	Yes	Yes	No	No	Yes	Yes
13050	16	24.16	24	20/20	20/20	0	1 (1/0/0)	Yes	Yes	No	No	No	No
13080	17	26.12	26.07	20/34	20/36.8	1 (0/0/1)	3 (1/0/2)	No	No	Yes	Yes	Yes	No
13045	19	25.36	25.7	20/20	20/20	1 (1/0/0)	5 (4/0/1)	No	No	Yes	Yes	No	No
13122	19	24.18	23.99	20/32	20/20	11 (9/1/1)	7 (5/0/2)	Yes	Yes	Yes	No	No	No
13100	20	23.61	23.66	−20.05dB‡	−17.99dB‡	9 (7/1/1)	8 (7/1/0)	No	No	No	No	No	No
13013	21	23.74	23.82	20/25	20/20	3 (3/0/0)	4 (3/0/1)	No	No	No	No	No	No
13020	21	24.56	24.55	§	§	0	0	No	No	No	No	No	Yes
13048	21	23.81	23.07	20/20	20/20	3 (2/0/1)	3 (3/0/0)	Yes	Yes	No	No	No	No
13039	22	25.44	25.4	§	§	3 (3/0/0)	2 (2/0/0)	No	No	No	Yes	No	No
13064	24	26.43	26.11	20/1000	20/30	0	0	No	No	No	No	No	No
13040	25	26.17	¶	20/25	¶	0	¶	No	¶	No	¶	No	¶
13130	27	25.09	24.83	20/30.6	20/23	8 (8/0/0)	7 (6/1/0)	Yes	Yes	Yes	Yes	No	Yes
13106	28	24.21	23.83	20/21	20/29.2	8 (6/1/1)	7 (6/0/1)	Yes	Yes	No	No	No	No
13057	30	26.55	26.74	20/25	20/25	3 (2/0/1)	3 (3/0/0)	No	No	Yes	Yes	Yes	No
13071	30	23.77	23.84	20/40	20/40	1 (1/0/0)	1 (1/0/0)	No	No	No	No	No	No
13125	30	23.4	23.27	20/40	20/40	8 (6/1/1)	9 (7/1/1)	Yes	Yes	Yes	Yes	No	No
13017	31	§	§	20/25	20/25	0	0	No	No	No	No	Yes	No
13132	32	25.02	24.61	20/800	20/24	0	6 (5/1/0)	No	No	No	Yes	No	No
13005	34	25.09	25.58	20/27.8	20/200	3 (2/0/1)	0	No	No	No	Yes	Yes	No
13051	34	23.24	23.48	20/27.8	20/44	3 (1/2/0)	6 (4/0/2)	No	No	Yes	Yes	Yes	Yes
13131	35	24.9	24.76	20/26.4	20/27.8	8 (6/0/2)	8 (6/0/2)	No	No	Yes	Yes	Yes	Yes
13070	41	25	24.99	§	§	0	0	No	No	No	No	No	No
13118	43	22.57	22.54	20/30	20/23	6 (5/1/0)	8 (6/2/0)	No	No	No	No	No	No
13126	44	24.29	24.09	−32.77dB‡	−32.75dB‡	2 (2/0/0)	5 (2/2/1)	No	No	No	No	No	No
13061	45	26.34	26.28	20/100	20/40	0	0	No	No	Yes	No	Yes	Yes
13068	45	24.98	25.29	20/25	20/200	5 (4/0/1)	2 (1/0/1)	No	No	No	No	Yes	No
13144	45	25.51	25	20/50	20/100	0	1 (1/0/0)	No	No	Yes	Yes	No	No
13076	46	§	24.22	20/200	20/60	0	0	No	No	No	No	Yes	Yes
13004	47	26.56	26.85	20/20	20/16	2 (2/0/0)	4 (2/0/2)	No	No	No	No	No	No
13015	47	24.64	24.94	20/100	20/50	0	0	No	No	No	No	Yes	Yes
13027	48	25.83	26.03	20/25	20/200	2 (1/0/1)	0	No	No	No	No	Yes	No
13062	48	27.9	27.5	§	§	0	0	No	No	No	No	Yes	Yes
13053	49	26.53	§	§	§	0	0	No	No	No	No	Yes	No
13117	49	24.91	24.78	HM	20/150	0	0	No	No	No	No	Yes	Yes
13102	50	26.08	¶	−32.7dB‡	¶	0	¶	No	¶	No	¶	No	¶
13063	53	25.38	¶	§	¶	0	¶	No	¶	No	¶	No	¶
13136	53	25.84	25.42	20/24	20/55.2	2 (1/1/0)	3 (1/0/2)	No	No	No	No	Yes	No
13012	54	23.92	24.1	20/50	20/230	0	0	No	No	No	No	Yes	Yes
13073	54	24.03	24.17	20/80	20/200	0	0	No	No	No	No	No	No
13032	55	24.18	23.89	20/160	20/42	2 (1/0/1)	1 (1/0/0)	No	No	Yes	Yes	No	Yes
13055	55	25.68	25.91	§	§	0	0	No	No	No	No	No	No
13034	56	24.48	25.03	20/44	20/176	0	0	No	No	No	No	No	No
13074	56	25.72	25.75	20/25	20/70	1 (1/0/0)	0	No	No	No	No	Yes	Yes
13019	57	22.34	22.22	§	§	0	0	No	No	No	No	No	No
13067	58	25.45	25.71	20/42	20/33.6	0	0	No	No	No	No	No	No
13078	58	25.84	24.61	20/200	<20/200	0	0	No	No	Yes	No	No	No
13066	60	22.76	22.92	20/30	20/30	0	0	No	No	Yes	No	Yes	No
13086	60	24.56	24.84	§	§	0	1 (1/0/0)	No	No	No	No	Yes	Yes
13075	61	25.2	25.5	20/50	20/200	0	0	No	No	No	No	No	No
13072	62	25.18	25.59	20/30	20/60	1 (1/0/0)	1 (1/0/0)	No	No	No	No	No	No
13101	62	24.74	25.03	20/80	20/27.8	1 (0/0/1)	0	No	No	No	No	No	No
13087	63	27.01	27.03	§	§	0	0	No	No	No	No	No	No

TABLE 1. Continued

OD, right eye; OS, left eye.
* Number of retinal locations at which cone density was measured (number of locations where cone density was within/higher/lower than the normal range reported by Song et al. ³²).
† Presence (yes) or absence (no) of each feature in AOSLO images for each eye.
‡ Visual acuity data not available, but Humphrey Visual field data was available. Central 30-2 Threshold Test, Mean Deviation dB.
§ Data not available.
Remaining central island of intact retina was less than 0.5 mm in radius along all four meridians.
¶ Eye was excluded from the study.

ment of retinal structure phenotype and progression of disease. This study uses the above named high-resolution ophthalmoscopy techniques to observe retinal structure in patients affected by and carriers of choroideremia.

METHODS

The research presented in this study was approved by the institutional review board at the University of Pennsylvania and the Children's Hospital of Philadelphia, and followed the tenets of the Declaration of Helsinki. All light exposures adhered to the maximum permissible exposure limits set by the American National Standards Institute standards.²³ Following explanation of the study requirements and potential risks, subjects gave informed consent and voluntarily enrolled in the study. Children age 7 to 17 gave assent and had written parental permission before enrollment.

Patients diagnosed with choroideremia, carriers of choroideremia, and normal sighted control subjects were enrolled in this study. In total, 111 eyes of 57 choroideremia patients age 7 to 63, 33 eyes of 18 choroideremia carriers age 9 to 68, and 16 eyes of 8 normal sighted control subjects were included in the study. Table 1 (patients), and Supplementary Tables S1 and S2 (controls and carriers) list the patients and eyes included in the study. Three choroideremia eyes and three carrier eyes were excluded from analysis, either because the patient was unable to fixate reliably (patient 13102), or AOSLO images had optical artifacts (a bright stationary back-reflection) in the images that precluded analysis (patients 13040, 13041, 13049, 13083). The cause of the back reflection is unclear; however, patient 13040 had undergone previous eye surgery, and carriers 13041, 13049, and 13083 were all hyperopic. The left eye of patient 13063 was not imaged due to time constraints. Study eyes of participants were dilated using phenylephrine hydrochloride (2.5%) and tropicamide (1%). Subjects then were imaged using AOSLO, SLO, AF, and OCT techniques. Axial lengths for all subjects were recorded using a Zeiss IOLMaster (Table 1; Supplementary Tables S1, S2; Carl Zeiss Meditec, Jena, Germany).

Spectral domain OCT and en face SLO imaging was performed using the Heidelberg Spectralis system (Heidelberg Engineering GmbH, Heidelberg, Germany). Infrared images of the fundus and AF images of the RPE layer were recorded using the SLO mode of the Spectralis. Cross-sectional OCT images along the horizontal and vertical meridians through the central fovea were recorded.

The AO images were obtained with an AOSLO manufactured by Canon, Inc. (Tokyo, Japan). The technical details of the AOSLO system used for this study have been published previously.^{24,25} Wavefront sensing for this system was done using a superluminescent diode at 760 ± 5 nm over a 4-mm pupil. Aberration correction was performed using two liquid crystal on silicon spatial light modulators to modulate the phase of the wavefront sensing beacon in two orthogonal polarization directions. The wavefront sensor and liquid crystal modulator was controlled by custom software in a closed loop

fashion until the root-mean-square wavefront error fell below $0.04 \mu\text{m}$. Following this, the wavefront sensing beacon was turned off and the SLO imaging light (superluminescent diode at 840 nm with a full width at half maximum of 50 nm) and scan system was turned on. Thus, AO aberration correction was static during imaging, and wavefront sensing and imaging were performed in sequence. The imaging beam diameter was 4 mm entering the pupil. This system configuration provides a theoretical resolution limit of $5 \mu\text{m}$, which is sufficient for imaging the cone photoreceptors 0.5 mm and further eccentric from the fovea, as has been demonstrated in a large population of normal controls.²⁵

The patient was instructed to hold their head steady using a forehead rest and chin rest, and to fixate at a white cross within the system. By adjusting the location of this fixation cross, AO images at two different magnifications were taken at numerous different retinal locations within 2.5 mm of fixation. The larger field (~ 1.7 mm) was used for precise alignment of the AOSLO images to the other imaging modalities. The small field (~ 0.34 mm) depicted the high-resolution AO images of the fundus – only these small field images allowed for observation of the cone photoreceptors and, thus, only the small field images were used for cone mosaic analysis. During AOSLO imaging, retinal videos were recorded for 1 second at 32 frames per second. Multiple 1-second videos were recorded at each retinal location to maximize the likelihood of successful imaging.

The AOSLO images were post processed using a custom designed software package from Canon, Inc. A reference frame was chosen automatically by calculating the relative offset between neighboring frames of the video, and choosing the frame that most overlaps with the other frames and that does not have large distortions from intraframe eye motion. The 32 frames recorded for each image series were cross-correlated and averaged to produce a final intraframe registered image that also removed warping from the sinusoidal scan. These final averaged images then were exported from the Canon software and manually aligned to the Spectralis SLO and OCT images for each patient using Photoshop (Adobe Systems, Mountain View, CA, USA). Every AOSLO postprocessed image was reviewed at least twice by author JM to categorize and determine the prevalence of structural features observed in choroideremia. The scale for each AOSLO image was determined by the scan size assuming a 24-mm axial length and then multiplied by the ratio of the patient's axial length to 24 mm.

In areas showing a contiguous cone mosaic at retinal eccentricities between approximately 0.5 and 1.5 mm from the fovea, we used a semiautomated Matlab program (Mathworks, Natick, MA, USA) to measure cone density.^{26,27} Cone densities were measured and plotted only for images along the vertical and horizontal meridians in which the full cone mosaic was clearly visible (determined during the image review process by author JM). Images were considered only to be of sufficient quality to allow cone counting if it appeared that all cones in the mosaic were visible. The AOSLO images of the cone mosaics were cropped to a square region of interest $80 \mu\text{m}$ per side. As a first pass, the cones were selected automatically within this region of interest using the Matlab script. Following

FIGURE 1. Retinal images from the right eye of choroideremia patient 13092. (A) The IR fundus SLO imaging shows retinal atrophy in the periphery with scalloped edges along the border. (B) Hypo-AF reveals areas of degenerated RPE at these same locations with centrally intact RPE depicted by remaining AF. The *white arrows* on the IR fundus image (A) give the horizontal location of the OCT B-scan (C) through the central retina. Centrally, the laminar structure of the retina remains intact, though the RPE and interdigitation layer are thinned and difficult to distinguish. The *yellow arrows* mark the abrupt transition area between the central retina and area of atrophy. (D) The montage of larger field of view AO images depicts the central retina within approximately 1.5 mm of fixation in all directions. Numerous hyper-reflective clumps can be observed throughout this region. *Colored boxes* show the locations for each high-resolution AO image given in Figure 2.

FIGURE 2. The AO images from the right eye of patient 13092 at the locations identified by *colored boxes* in Figure 1. The cone photoreceptor mosaic is intact and continuous at each location. The cone density for each location is given on each image, each of these cone densities is within the previously reported normal range given by Song et al.³² Clumps of hyper-reflective cones are readily observed at locations 3, 4, and 5. Locations 1, 2, 3, and 5 are included in Table 1 and the plot of cone density versus eccentricity (Fig. 6). Locations 4, 6, and 7 are not included in Table 1 or Figure 6, because the locations are not along the either horizontal or vertical meridian. *Scale bar*: 50 µm.

FIGURE 3. Retinal images from the left eye of choroideremia patient 13045. (A) The IR fundus SLO imaging shows retinal atrophy, which has progressed further centrally as compared with 13092. Large choroidal vessels can be seen in the periphery with the dark central region showing remaining central retina. (B) The AF imaging was difficult in this patient but the contrast enhanced image shown does reveal remaining AF in the central retina corresponding to the dark central region on IR imaging. The *white arrows* on the IR image (A) give the horizontal location of the OCT b-scan (C) through the fovea. The OCT shows outer retinal tubulation temporal to the central intact retina (*yellow arrow*). The *orange arrowheads* show interlamellar bridges similar to those described by Jacobson et al.⁶ These bridges mark the abrupt transition from central relatively intact retinal lamination to outer retinal atrophy. *Blue arrowhead* points to inner retinal microcysts as previously described.^{22,30} The *red arrowheads* mark the abrupt border to the outer nuclear layer plus Henle's fiber layer, the external limiting membrane, and the ellipsoid band. The interdigitation band is indistinguishable from the RPE throughout the entire central region and the interdigitation band/RPE combination is thin compared to normal. (D) The montage shows the larger field of view AO images within the central 2 mm of fixation, showing the relatively centrally intact retina and surrounding atrophic area. *Colored boxes* show the locations for each high-resolution AO image given in Figure 4.

automated selection of the cone photoreceptors, images then were manually reviewed (by author JM) and corrected for any erroneous cone counts (either omissions or additions) by the automated process. Cone density then was calculated using a square 55 μm per side in the center of the region of interest to eliminate any edge artifacts.

RESULTS

The SLO IR imaging revealed the typical scalloped edges of the RPE in patients with choroideremia. As expected, choroideremia patients demonstrated peripheral degeneration early in the course of the disease. In degenerated areas of RPE and retina, large intact choroidal vessels could be observed. The AF imaging showed hypo-AF in areas of degeneration, while central AF remained present. Preserved AF corresponded with

central relatively spared retina. The OCT images showed relatively centrally intact laminar structure for the choroideremia patients; however, the RPE layer was thinned and the interdigitation zone was absent or indistinguishable from the RPE layer. The ellipsoid band and outer nuclear layer plus Henle's fiber layer was maintained over these areas. At the border between central retina and atrophy, choroidal backscatter increased and the OCTs showed abrupt loss of outer retinal layers at this same location in most cases. Rarely, the outer retinal layers tapered off more peripherally than the location marking the increase in choroidal backscatter. The OCT B-scans showed outer retinal tubulations,^{28,29} interlamellar bridges,⁶ and inner retinal microcysts^{22,30} as previously described. Figures 1 to 4 show montage images from all imaging modalities for choroideremia patients 13092 and 13045, respectively.

FIGURE 4. The AO images from the left eye of patient 13045 at the locations identified by colored boxes in Figure 3. The central AO images show the cone mosaic is intact and continuous throughout this region. The AO images outlined in *red* and *yellow* (locations 4 and 6) show retinal locations at the border of atrophy. The cone mosaic is intact and visible up to the edge of the atrophic region. The AO image outlined in *orange* (location 5) shows the choroidal blood vessel network, visible because of the atrophic retina at this location. The cone mosaic is not observed at this location. Locations 1, 2, 3, 4, and 6 are included in Table 1 and the plot of cone density versus eccentricity (Fig. 6). Scale bar: for AOSLO images outlined in color, 50 μ m.

Cone Photoreceptor Imaging and Mosaic Characteristics

The AOSLO imaging revealed numerous features in choroideremia patients. Similar to controls, a contiguous cone photoreceptor mosaic can be observed in the AOSLO images in areas of centrally intact retina excluding the fovea (Figs. 2, 4). Figure 5 shows the cone mosaic of several patients and carriers of choroideremia in comparison with normal sighted controls. Though the images vary in brightness and contrast, the cone mosaic is visible and continuous throughout the image and cone density was measured in each of these examples (Supplementary Fig. S1).

Table 1 shows the number of retinal locations used for measuring cone density in each eye and Figure 6 plots the measured cone densities as a function of retinal eccentricity. As stated above, we only measured cone density at locations where the image showed the full cone mosaic between 0.5 and 1.5 mm from fixation along the meridians. We measured cone density in at least one location for 59 eyes of 34 patients (Table 1). We were unable to measure cone density in 33 eyes because the remaining island of central intact retina was less than 0.5 mm in all directions. A total of 16 eyes had central islands of intact retina extending less than 0.5 mm from fixation in most directions with insufficient quality to measure cone density at the remaining 0.5-mm border location(s). Two eyes (13050, right eye and 13031, left eye) had clumps of highly reflective cones that precluded accurate measures of density. For unclear reasons, image quality was not sufficient for measuring cone density in patient 13040, right eye.

Figure 6 shows the cone densities of our patients in comparison with our normal sighted controls and previous reports in the literature.^{25,31,32} The majority (141 of 163, 86.5%) of our measurements of cone density in choroideremia patients from approximately 1.0 to 1.5 mm remained within the range of cone densities previously reported by Song et al.³² for normal control subjects. We measured cone densities higher than (though close to) the previously reported normal range from Song et al.³² in 19 of 163 measurements of

choroideremia cone density from approximately 1.0 to 1.5 mm eccentricity (Fig. 6). At 0.5 mm eccentricity, cone density was either within normal limits or was reduced from normal particularly in the superior and nasal meridians (Fig. 6). Table 1 shows the number of measurements within, above, and below the normal limits of previously reported cone density for each patient.

Though cone density was largely within the previously reported range of normal cone densities, abnormalities in the cone mosaic were present. The cone mosaic image quality in choroideremia patients was inferior to that of normal controls and choroideremia carriers (Fig. 5); image contrast was lower in patient images (Fig. 5, patients 13051 and 13032) and cone edges were less well-defined (Fig. 5, patients 13122 and 13057). In addition, other cone mosaic abnormalities were observed despite normal cone density. In 18 eyes of 9 patients (Table 1), the cone mosaic showed groups of cones with relatively high reflectance relative to the surrounding cone mosaic, similar to the mosaic of choroideremia patient 13092 shown in Figure 2. The hyper-reflective clumps of cones were observed only in patients age 30 and younger, and were observed in half of the choroideremia patients less than 20 years of age (Table 2).

Boundary Between Central Intact Retina and Atrophy

The AOSLO imaging led to the observation that the cone mosaic remains intact up to the border of retinal atrophy (Fig. 7). We verified these findings by looking at RPE and photoreceptor loss at the same locations in OCT and AF images. The AOSLO images in Figure 7 show the cone mosaic abruptly ends at the border of the atrophic retina for these locations. The IR and AF images show hyper-reflectance and hypo-AF at this same edge, corresponding with loss of the RPE at these locations. The OCTs at these same locations show a sharp increase in choroidal backscatter, loss of the RPE layer, interdigitation layer, ellipsoid layer, and outer nuclear layer plus Henle's fiber layer at the atrophic border.

The AOSLO image in Figure 8 shows an example where the cone mosaic appears to extend beyond the border of functional RPE. The IR and AF images at these locations show hyper-reflection and hypo-AF, which is consistent with depigmentation and functional loss of the RPE, respectively. The OCT aligned to the AOSLO image in Figure 8 shows an increase in choroidal backscatter, thinned RPE, loss of the interdigitation layer, disrupted but present ellipsoid layer, and intact outer nuclear layer plus Henle's fiber layer. Further evidence (beyond the OCT comparison) that the reflections observed in the AOSLO image may be the cone mosaic rather than other reflective structures include: The reflections are regularly spaced and of similar size, shape, and density as expected for cones at this location; and the choroidal vessels are not observed at these locations as they are in the AOSLO areas corresponding to cone loss and RPE atrophy in Figure 7. These data suggest that at this location, the RPE is undergoing degeneration before the cones.

Disease Features in Atrophic Retina

Beyond the border of RPE degeneration, choroidal blood vessels and blood flow are visible in the AOSLO images from 56 of 57 choroideremia patients (all except 13092). Figure 9 shows examples of the choroidal vessels observed in AOSLO images, and the corresponding IR and OCT images at these same locations. These vessels only become visible in locations corresponding to retinal atrophy. Choroidal blood flow and vessels are not observed in AOSLO images of normal sighted

FIGURE 5. High-resolution AO images of the cone mosaic at approximately 1 mm nasal and 1 mm temporal in normal control subjects (NCS), carriers of choroideremia (CAR), and choroideremia patients (CHM). Cone densities for each of these images fell within the previously reported normal range (Supplementary Fig. S1).³² Though cone density is within the range of normal cone densities for each of the choroideremia patient images shown here, the cones appeared less reflective and with lower contrast in patient images compared to normal and choroideremia carriers. Scale bar: 20 μ m.

FIGURE 6. Plot of cone density versus eccentricity along each meridian of the retina. *Open triangles* are choroideremia patient data, *open circles* are choroideremia carrier data, *pluses* are normal control data. *Solid black line with closed circles* is replotted histology data from Curcio et al.³¹ *Solid gray lines* are the previously reported range of cone densities measured with AOSLO replotted from Song et al.³² *Solid squares with error bars* are the previously reported normal densities from Park et al.²⁵ The data from our eight normal controls match previously reported control data, thus showing the AOSLO used in this study is capable of resolving the cone mosaics at these retinal eccentricities. S, superior; I, inferior; N, nasal; T, temporal.

control eyes because any signal from the choroid is blocked by the overlying RPE and photoreceptor layers. The AOSLO images of 13092 did not show the choroidal vessels, only because AOSLO images were acquired over the central 1.5 mm where the central retina remained intact for this patient (Fig. 1).

Several other distinct features also can be found using AOSLO imaging beyond the border of RPE and retinal atrophy in choroideremia patients. We have termed these features “bubble-like” (previously undescribed) and “inner retinal microcysts” as described by Syed et al.²² The bubble-like feature (Fig. 10) was seen in at least one location imaged with AOSLO in 34 eyes of 23 patients with choroideremia (Table 1). The bubble-like feature appeared as hyper-reflective spots with dark edges. Precise alignment of OCT images to AOSLO images show the bubble-like features on AOSLO co-locate with hyporeflective spots in the choroid in OCT. These features can be observed on IR SLO without AO and clearly align to hyporeflective space in the choroid on OCT (Fig. 11). One possibility is that the hyper-reflection of these spots en face may originate from increased scleral backscatter, with the dark edges being the choroidal remnants co-located with the hyporeflective space on OCT. Supplementary Video S1 shows

bubble-like features in an area of atrophy adjacent to the central intact retina. Adjusting the focus of the AO shows that the bubble-like features were in focus approximately 0.4 diopters (D) posterior to the photoreceptor layer in the adjacent retina, thus giving more evidence that these features are located deeper than the photoreceptors. Bubble-like features did not align with outer retinal tubulations or inner retinal microcysts on OCT.

The inner retinal microcysts (Fig. 12) were seen in at least one location imaged with AOSLO in 26 eyes of 17 choroideremia patients (Table 2). Corresponding OCT images show these inner retinal microcysts correspond to hypo-reflective space anterior to the photoreceptor layer in the inner retina, as previously described²² (Fig. 12). Inner retinal microcysts on AOSLO did not align with outer retinal tubulations or choroidal features on OCT.

Of 111 eyes with choroideremia studied, 50 exhibited either bubble-like features and/or inner retinal microcysts. The incidence of both of these features likely is higher, as the AOSLO images only covered a portion of the retina and the feature must have been clearly visible in the AOSLO image to be tabulated. While both features were observed in all age groups (Table 2), the inner retinal microcysts were most

FIGURE 7. The AOSLO, IR, AF, and OCT images showing border regions of retinal atrophy, where the cone mosaic abruptly ends at the same location as the border of RPE atrophy (marked with an *asterisk* in the AOSLO images). The AF images show preserved AF in locations corresponding to intact RPE and cone mosaics in AOSLO. The OCT shows an abrupt end to the outer nuclear layer plus Henle's fiber layer, the ellipsoid band, and the RPE with increased choroidal backscatter at the border of atrophy, consistent with hyper-reflection at the same location on IR. Again the interdigitation band cannot be distinguished from the thinned RPE. The *white squares* on IR and AF images show the location of the AOSLO image. *Arrow* on IR image shows the location and direction of the OCT line.

prevalent in the 30 to 39 age group, while the bubble-like features were most prevalent in the 40 to 49 age group.

Choroideremia Carriers

Similar to normal controls and choroideremia patients, cone photoreceptors are visible in images from carriers of choroideremia. Figure 13 shows a montage of all imaging modalities from choroideremia carrier 13036. Cone density was measured in retinal locations showing complete and contiguous cone mosaics as described above (Fig. 6; Supplementary Table S2). Some retinal locations exhibit local regions of cone loss resulting in a patchy cone mosaic (Fig. 14). These regions of cone loss in AO images correspond with patchy locations of RPE loss as seen with hypo-AF on AF imaging and hyper-reflection on IR imaging. These findings agree with other reports of AF and visual function in choroideremia carriers.^{33,34} Other features visible in choroideremia patients are viewed occasionally in images from carriers as well, though the

frequency is greatly reduced. The left eye of choroideremia carrier 13030 had a single bubble-like feature visible in the AOSLO image at approximately 1 mm temporal. The right eye of 13046 had inner retinal microcysts visible at approximately 1.5 mm nasal. Choroideremia carrier patient 13123 was symptomatic of choroideremia with skewed X-inactivation, exhibiting scalloped edges of retinal atrophy encroaching on the macula and thinned RPE with an indistinguishable interdigitation layer on OCT (Supplementary Fig. S4). Patient 13123 also exhibited inner retinal microcysts in both eyes. Choroideremia carriers also could exhibit outer retinal tubulation on OCT (Supplementary Fig. S4).

DISCUSSION

RPE Degeneration as a Primary Site of Disease

One goal of the present study was to determine which retinal layers are primary sites of degeneration in patients with

FIGURE 8. The IR (A), AF (B), and OCT (C) images from patient 13071, right eye. The IR image (A) shows central area of remaining retina, with scalloped edges at the border of the atrophic region. The AF image (B) shows the central retinal area still retaining AF and, thus, still containing functional RPE. (C) The OCT image shows the central laminar structure of the retina. The interdigitation layer is difficult to distinguish from the RPE/Bruch's membrane layer; however, the combination of these two layers become thinner at the location marked by the *red arrow* and slight increase in choroidal backscatter. A second increase in choroidal backscatter is observed at the *orange arrow*. The ellipsoid band is present beyond (further nasal than) the *orange arrow* and the ellipsoid band is disrupted though visible at the yellow arrow. The *blue arrow* marks the edge of the outer nuclear layer plus Henle's fiber layer. The wide extent of horizontal locations marking the loss of the different outer retinal layers on OCT in this eye, is different from the sharp borders of atrophy shown in the OCTs of Figures 1, 3, and 7. (D) The OCT image magnified by a factor of 3 (area outlined by the black rectangle in [C]). (E) The AOSLO image of the cone photoreceptors at the retinal location outlined by the *white box*. Evidence that the reflections seen in the AOSLO image at this location are due to the presence of cones is as follows: The cone mosaic is visible at the same focus level throughout the entire image. The reflections are similar in size, shape, pattern, and density as what would be expected for the cone mosaic at this location. The OCT images show the ellipsoid band is present though disrupted. Evidence that the RPE may be a primary site of disease at this location is as follows: The increase in choroidal backscatter at this location shows that the RPE is at minimum less pigmented than locations further central. The less pigmented RPE also is observed in the IR image at this location. The AF is not present at this location, suggesting at minimum that the RPE is not functioning normally.

TABLE 2. Incidence of AOSLO Image Features by Age

Age	Total*	Hyper-Reflective Spots†	Inner Retinal Microcysts†	Bubble-Like Structures†	None‡
	# Eyes/ # Patients	# Eyes/# Patients/ % Eyes	# Eyes/# Patients/ % Eyes	# Eyes/# Patients/ % Eyes	# Eyes/# Patients/ % Eyes
<20	18/9	10/5/55.56%	6/4/33.33%	3/2/16.67%	4/2/22.22%
20–29	17/9	6/3/35.29%	3/2/17.65%	2/2/11.76%	9/6/52.94%
30–39	16/8	2/1/12.5%	10/6/62.5%	7/5/43.75%	4/3/25%
40–49	26/13	0/0/0	3/2/11.54%	13/8/50%	11/7/42.31%
50–59	22/12	0/0/0	3/2/13.64%	6/4/27.27%	14/9/63.64%
>59	12/6	0/0/0	1/1/8.33%	3/2/25%	9/5/75%
Total	111/57	18/9/16.22%	26/17/23.42%	34/23/30.63%	51/32/45.95%

* Total number of eyes/total number of patients per age group.
† Number of eyes/number of patients/percent of eyes with each feature in AOSLO images per age group.
‡ Number of eyes/number of patients/percent of eyes without hyper-reflective spots, inner retinal microcysts, or bubble-like structures in AOSLO images per age group.

FIGURE 9. The AOSLO images depicting choroidal vessels in locations corresponding to retinal atrophy in choroideremia patients. The cone mosaic is not observed at these locations. Corresponding IR and OCT images are shown at the same locations. The *white squares* on the IR images show the locations of the AOSLO images. The *arrows* on IR images show the locations and directions of the OCT lines.

FIGURE 10. Examples of the bubble-like features observed in the AOSLO images of a subset of choroideremia patients. These features are observed only over regions corresponding to retinal atrophy; the cone mosaic is not observed at these locations. The bubble-like features can be observed in the IR images. The *white box* in the IR image outlines the area marked by the AOSLO image above. The *arrows* on IR images show the locations and directions of the OCT lines. The OCTs at the same locations show the bubble-like features co-locate with hyporeflective space in the choroid (*white arrowheads*). The OCT of patient 13086 shows there are no inner retinal microcysts and that the bubble-like features do not co-locate with inner retinal cysts. The OCTs from patients 13012, 13057, and 13086 show the bubble-like features do not co-locate with interlaminar bridges. Supplementary Video S1 also shows the features are located posterior to the photoreceptors.

FIGURE 11. The IR and OCT images from patient 13062. The IR image shows numerous clearly visible bubble-like features spread throughout the atrophic macula. Four OCT lines are shown in precise alignment with the IR image. The bubble-like features outlined in red, yellow, white, and blue boxes on the IR image correspond to the red, yellow, white, and blue boxes on each OCT line. The bubble-like features within each of these boxes co-locate with hypo-reflective space in the choroid (white arrows on OCT). A different retinal feature (teal arrow on IR and OCT) was used to verify the alignment of each OCT line on the IR image separate from the bubble-like features. The bubble-like features do not align with inner retinal microcysts, interlaminar bridges, or outer retinal tubulations. A different retinal feature (teal arrow on IR and OCT: OCT line 1, pigment clump; OCT line 2, outer retinal tubulation; OCT line 3, retinal blood vessel; OCT line 4, retinal perforation) was used to verify the alignment of each OCT line on the

choroideremia. For this we carried out a multimodality imaging study on a large number of affected individuals and carriers. Similar to other studies,^{2,5,8–10,35} this study presented data to indicate the RPE is a primary site of degeneration for choroideremia. This is evident by aligning and comparing images between relatively intact retina and the border of retinal atrophy across the various imaging modalities. Alignment of the IR, AF, and AOSLO en face imaging modalities is precise (within 20 μm on the IR image). Alignment of the OCT to the IR image is limited by placement of the OCT line on the IR image by the Spectralis system, which then can be fine-tuned manually. We estimate this error is less than 100 μm .

The OCT shows that the first retinal layer to disappear in these patients is the interdigitation layer between the RPE and photoreceptors. Even within the relatively centrally intact remaining retina, the interdigitation layer is indistinguishable from the RPE layer, while the ellipsoid band, external limiting membrane, and outer nuclear layer plus Henle's fiber layer remain intact. The AOSLO imaging at the border of the atrophic region provides three different sets of evidence that RPE is one primary site of degeneration in choroideremia. First, we showed an example where RPE AF is lost while cones are retained (Fig. 8). Lazow et al.⁹ suggest it may be possible for the cone cells to survive for a period of time without a detectable outer segment layer, and, similarly here, we found that the cones appeared to linger even following loss of the interdigitation layer and abnormal RPE. It is possible that cones at this location are in the process of degeneration, and, thus, sequential imaging might show subsequent loss in the photoreceptor layer.

Second, we observed numerous locations where the cones are present up to the edge of the atrophic RPE border and where OCT shows sharp loss of outer retinal layers (Figs. 1, 3, 7). It follows that the cones here are surviving in the retinal locations where RPE remains functional. Third, we did not observe the underlying RPE mosaic in areas of cone loss, as has been reported in other AOSLO imaging studies for inherited retinal degenerations caused by primary photoreceptor defects, such as in cone rod dystrophy³⁶ and Stargardt retinal dystrophy.¹⁶

Cone Involvement in Choroideremia

Though it is unlikely that the RPE is fully healthy in the central most retina,⁸ the RPE does retain AF, as would be expected in RPE that can support the overlying photoreceptors. Nevertheless, we observed abnormalities in the cone mosaic images, including decreased image contrast, decreased reflectivity, and clumps of bright cones. Cone density generally was within the previously reported normal range at 1.0- and 1.5-mm eccentricities.³² However, 43.0% of our measurements showed cone density is reduced at 0.5 mm eccentricity (Fig. 6, Table 1). Of interest, we found a higher percentage of patients exhibiting cone density loss at 0.5 mm nasal (73.7%) and 0.5 mm superior (61.5%) than the temporal (22.7%) and inferior (10.5%) meridians. Similarly, Lazow et al.⁹ found more loss in nasal retina than temporal retina in 7 choroideremia patients, and Jacobson et al.⁶ report photoreceptor loss and remodeling in young choroideremia patients in the parafoveal region. Sequential imaging, particularly at the border of atrophy, of photoreceptor structure throughout disease progression might help document the mechanism causing the observed pattern of retained and lost cone structure found in the present study.

IR image separate from the bubble-like features. The bubble-like features do not align with inner retinal microcysts, interlaminar bridges, or outer retinal tubulations.

FIGURE 12. Examples of inner retinal microcysts observed in the AOSLO images of a subset of choroideremia patients. These features are only observed over regions corresponding to retinal atrophy; the cone mosaic is not observed at these locations. The inner retinal microcysts cannot be readily identified in the IR images. The *white box* in the IR image outlines the area marked by the AOSLO image. The *arrows* on IR images show the locations and directions of the OCT lines. OCTs at the same locations show the features co-locate with hyporeflective space in the inner retina (*white arrowheads*).

Though the AOSLO used for this study is not capable of imaging the rods, AO imaging of rods is possible.³⁷ It would be of interest to image the rods in these patients, especially given that previous studies of choroideremia have shown that the rods are affected earlier than the cones.⁷

The pattern of cone loss at 0.5 mm, but generally normal density at 1.0 and 1.5 mm, was unexpected, as were the few data points at 1.5 mm where cone density was measured above the normal range of previously measured cone densities (Fig. 6). Though unexpected, this pattern also was observed by Syed et al.²² in a subset of choroideremia patients. Because cone detection and selection in AOSLO images can be difficult due to other reflective retinal features, we took a conservative approach to measuring cone density; cone density was measured only in retinal locations where the AOSLO image showed an intact cone mosaic at the same focus level throughout the entire image, where the IR and AF images showed central intact retina, and where the OCT image showed the ellipsoid band at the same location. Use of the IR, AF, and OCT images to help assess reflectance structure in the AOSLO image decreases the likelihood that reflective structures other than the cones are included in quantitative cone density analysis.

The OCTs at locations corresponding to reduced cone density show thinning and loss of the interdigitation band. Loss of photoreceptor interdigitation with the RPE as a primary event suggests two hypothesis regarding disease mechanism: the photoreceptor outer segments are first to degenerate or the RPE cells are compromised, in particular at the apical processes. In the first case, the photoreceptors would be a primary site of degeneration, while in the second, the RPE is the primary site of degeneration and the cone loss is secondary. Alternatively, both layers could be undergoing degeneration independently, making the photoreceptor layer and RPE

primary sites of degeneration in patients with choroideremia. Because the present study did not look RPE AF and degeneration on the cellular level,³⁸ we are unable to conclude whether the RPE cell mosaic within the central region at locations corresponding to reduced cone density is fully intact. Cellular imaging of the RPE using AF³⁸ or dark field³⁹ in combination with AOSLO imaging may help determine if there is partial RPE loss at these locations before the observed cone loss. The RPE imaging also may help determine if the reduced contrast in patient images, the hyper-reflectance clumps observed in the cone mosaics of half our patients younger than 20, and the low frequency hyper-reflectance structures observed in a single choroideremia patient by Syed et al.²² can be attributed to the RPE layer and/or interdigitation zone on OCT. Further study to investigate both cell types with single cell resolution³⁸ would help answer the question of whether cone photoreceptors and RPE cells are both primary sites of degeneration in choroideremia, or whether cones degenerate secondarily to the RPE.

Photoreceptor Structure Compared With Visual Function

The present study did not look in detail at visual function, and it would be interesting to use fundus guided microperimetry or another visual function procedure to test whether the remaining cones are fully functional. Though choroideremia patients exhibit significantly reduced peripheral vision and constricted visual fields, high visual acuity can be maintained until later decades of life (Table 1).³ Visual acuity is driven by foveal cone function, so the retention of visual acuity until the fifth decade of life suggests that at least some foveal cones remain intact and functional until late in the disease process. In the present study, the AOSLO system resolution precludes

FIGURE 13. The IR, OCT, AF, and AOSLO montage images of the right eye of choroideremia carrier 13036. The fundus shows patchy RPE atrophy in the IR (A) and AF (B) images. The white arrows on the IR fundus image (A) give the horizontal location of the OCT B-scan (C) through the central retina. The layer corresponding to the interdigitation zone is lost and the ellipsoid band is disrupted (yellow arrows) at locations corresponding to patchy atrophy on IR and AF. (D) The montage of the larger field of view AO images depicts the central retina within approximately 1.5 mm of fixation in all directions. Colored boxes show the locations for each high-resolution AO image given in Figure 14.

measuring foveal cone density; however, we do measure parafoveal cone density, and find that high numbers of cones remain present within the central macula. However, we do measure reductions in cone density from normal, in particular at 0.5 mm eccentricity. This also could be consistent with the reductions of visual acuities below 20/20 – only 13 of 111 eyes presented with visual acuity of 20/20 or better (Table 1), therefore, we hypothesize that, at a minimum, some cones in the fovea are nonfunctional. Foveal cone imaging using an AOSLO system capable of resolving the foveal cones⁴⁰ would determine if these cones are structurally present or if cone density is reduced at the fovea.

Disease Features in Atrophic Retina

Other features of interest, in particular bubble-like structures and inner retinal microcysts, were found in a substantial number of our patients (Table 1, Table 2) at locations corresponding with RPE and photoreceptor loss as seen on IR, AF, OCT, and AOSLO imaging. Though these features differ in appearance (Figs. 10, 12) they may be different stages on a continuum of the same degenerative process. Evidence for this is as follows: Occasionally, we observe the inner retinal

microcysts and bubble-like features in adjacent retinal locations in the same patient, and inner retinal microcysts are more prevalent in patients in their 30s, while bubble-like features are more prevalent in patients in their 40s (Table 2).

While the precise anatomic correlate of these shapes is unknown, we believe that the bubble-like features represent atrophy of the choroid, since OCT and AOSLO suggests that the features are deep and certainly posterior to the retinal vasculature (Figs. 10, 11, and Supplementary Video S1). Normal choroid consists of Bruch's membrane, choriocapillaris, Sattler's vascular layer, Haller's vascular layer, and the stroma with smooth muscle cells, collagen, and fibers.⁴¹ Mural cells,⁴² intercapillary pillars, mast cells, macrophages, and lymphocytes also are found throughout the choroid.⁴¹ Atrophy or disorganization of any of these layers and cell types could result in the bubble-like features observed in the AOSLO images and the hypo-reflective space in the OCT images of choroideremia patients. As well, Rodrigues et al.¹⁰ describe rosette-like structures in a 19-year-old patient with choroideremia along with atrophy in the RPE, Bruch's membrane, choriocapillaris, and inner and mid-choroid, chorioretinal adhesion, gliosis, and macrophage-like cells. Bonilha et al.⁵ describe giant lipophilic drops in the choroid of a 91-year-old symptomatic choroide-

FIGURE 14. Most of the AOSLO images show a complete and contiguous cone mosaic (locations 1, 3, 4, 6, and 7). The cone density for each location counted is given on each image, each of these cone densities is within the previously reported normal range given by Song et al.³² Some local disruption of the cone mosaic can be observed in locations 2 and 5 (asterisks on the AOSLO images). These locations also show local patches of hypo-AF corresponding to RPE degeneration (Fig. 13). Location 4 is not included in Table 1 or Figure 6, because the location is not along the either horizontal or vertical meridian. Scale bar: 50 μ m.

remia carrier, as well as clumps of collagen, and smooth and bristle-coated vesicular structures. Finally, Ghosh et al.⁴³ describe duplication of RPE and Bruch's membrane, collagenous tissue surrounding choroidal capillaries, clumps of fibrillar material, and a layer of collagen fibrils in histology images of two older patients with choroideremia.

Anatomically, the inner retinal microcysts may represent inner retinal changes that appear as hypo-reflective space on OCT (Fig. 12). Similar to the results of this study, Syed et al.²² identified inner retinal microcysts in choroideremia using OCT, and a previous OCT study of choroideremia by Genead and Fishman³⁰ found hyporeflective space, or cystic macular edema in the inner retina in 10 of 16 patients. In the present study, the OCTs corresponding to the inner retinal microcysts structures in AOSLO appeared similar to the inner retinal microcysts described by Syed et al.²² and the OCTs reported by Genead and Fishman.³⁰ Genead and Fishman³⁰ report an incidence of 62.5%, while the present study finds the inner retinal microcysts in 17 of 57 (29.8%) patients. However, AOSLO imaging only covered the central 2 mm of retina, therefore the incidence of these features likely is higher than what is reported in the present study. Regardless, the inner retinal microcysts and bubble-like structures are found in later stages of choroideremia disease and in locations of retinal atrophy where functional vision is already compromised.

Relevance to Therapeutic Intervention

With these results, therapies developed for choroideremia would certainly need to target the RPE and would ideally target photoreceptor cells as well. Retinal transduction of both cell types can be accomplished using AAV mediated gene delivery.^{44,45} As such, gene therapy is an attractive treatment for choroideremia, and a clinical trial for gene therapy of choroideremia is already underway with encouraging initial results.¹² Given that cones remain present despite loss of the interdigitation layer between the RPE and photoreceptors, treatments which preserve photoreceptor structure and function in the absence of RPE, or which deliver wild-type RPE in instances where the diseased RPE is or already has

degenerated also may be effective in delaying progression, maintaining, or restoring vision in patients with choroideremia. As well, treatments that maintain RPE cell structure and function could result in successful treatment for choroideremia by halting RPE cell degeneration and potentially delaying any independent photoreceptor degeneration. As clinical trials to treat choroideremia commence and continue, patient selection for trial inclusion and the determination of appropriate outcome measures will be critical to successfully monitor therapeutic effects. The AO imaging and other high-resolution imaging modalities could provide useful tools for predicting which patients may maximally benefit from a given therapy and also could provide quantitative, objective assessment regarding the ability of a treatment to retain or improve retinal structure and halt disease progression.

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