Quantitative Fundus Autofluorescence and Optical Coherence Tomography in Best Vitelliform Macular Dystrophy

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PURPOSE. Quantitative fundus autofluorescence (qAF), spectral domain optical coherence tomography (SD-OCT) segmentation, and multimodal imaging were performed to elucidate the pathogenesis of Best vitelliform macular dystrophy (BVMD) and to identify abnormalities in lesion versus nonlesion fundus areas.

METHODS. Sixteen patients with a clinical diagnosis of BVMD were studied. Autofluorescence images (30°, 488-nm excitation) were acquired with a confocal scanning laser ophthalmoscope equipped with an internal fluorescent reference to account for variable laser power and detector sensitivity. The grey levels (GLs) of each image were calibrated to the reference, zero GL, magnification, and normative optical media density, to yield qAF. Horizontal SD-OCT scans were obtained and retinal layers manually segmented. Additionally, color and near-infrared reflectance (NIR-R) images were registered to AF images. All patients were screened for mutations in BEST1. In three additional BVMD patients, in vivo spectrofluorometric measurements were obtained within the vitelliform lesion.

RESULTS. Mean nonlesion qAF was within normal limits for age. Maximum qAF within the lesion was markedly increased compared with controls. By SD-OCT segmentation, outer segment equivalent thickness was increased and outer nuclear layer thickness decreased in the lesion. Changes were also present in a transition zone beyond the lesion border. In subclinical patients, no abnormalities in retinal layer thickness were identified. Fluorescence spectra recorded from the vitelliform lesion were consistent with those of retinal pigment epithelial cell lipofuscin.

CONCLUSIONS. Based on qAF, mutations in BEST1 do not cause increased lipofuscin levels in nonlesion fundus areas.

Keywords: Best vitelliform macular dystrophy, bestrophin, lipofuscin, optical coherence tomography, quantitative fundus autofluorescence, retina, retinal pigment epithelium, scanning laser ophthalmoscope

Best vitelliform macular dystrophy (BVMD; also known as Best’s disease) is a rare disorder that is typically inherited in an autosomal dominant manner. Best vitelliform macular dystrophy is caused by mutations in BEST1 (formerly known as VMD2), a gene located on chromosome 11q131 that transcribes the bestrophin-1 protein located on the basolateral membrane of RPE cells.2 Ophthalmoscopic features of BVMD transcribes the bestrophin-1 protein located on the basolateral membrane of RPE cells.2 Ophthalmoscopic features of BVMD are not clear and eccentric vitelliform lesions can occur.3,4 Are usually restricted to the macula, although the reason for this is difficult to detect the voltage change, particularly in children.4 For a retinal disease such as BVMD, wherein histopathologic material is limited, optical coherence tomography (OCT) has been an invaluable tool to document disease pathology and progression. Studies using OCT have localized the vitelliform lesion to the subretinal space.6,10,11 Moreover, recent studies have reported thickening of the highly reflective band attributable to ensheathed cone outer segments (eOS12; previously referred to as cone outer segment tips, or COST13) in eyes with subclinical stage BVMD.10,14 However, this typically an abnormal electrooculogram (EOG),8 an electrophysiological test that measures changes in the transepithelial potential across the RPE.9 In BVMD, the voltage amplitude in response to light (light peak) is diminished as is the corresponding ratio between the light peak and dark trough (Arden ratio; >1.8 in healthy subjects). However, technical difficulties and the need for patient cooperation can make it difficult to detect the voltage change, particularly in children.4
thickening has not been quantified and compared with a larger group of healthy controls.

It has been reported,15-19 and it is generally assumed,5,20,21 that levels of lipofuscin in the RPE are increased in BVMD. Nevertheless, the mechanism linking bestrophin-1 functioning and a possible build-up of lipofuscin remains unexplained. Some reports of increased lipofuscin in BVMD have been based on nonquantitative assessment.15,16,18 In another case, measurements acquired from electron microscopic images led to the conclusion that the fraction of RPE cell volume occupied by lipofuscin granules was greater in BVMD versus non-BVMD eyes.17 Increases in the RPE of the patients ranged from 6 to 62 years (average 32.1 ± 18.8 years) and were similar to the 277 healthy subjects (mean 32.6 ± 18.8 years) and were similar to the 277 healthy subjects (mean 32.6 ± 18.8 years). Patients were staged based on fundus photographs and the family history, and a decreased Arden ratio on EOG. The latter test was performed according to International Society for Clinical Electrophysiology of Vision (ISCEV) standards.28

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Table. Summary of Demographic, Clinical, and Genetic Data

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Values in parenthesis indicate eyes without qAF images. BCVA, best corrected visual acuity.

METHODS

Patients

Included in this study were 16 patients with BVMD from 11 unrelated families. Demographic, clinical, and genetic data are presented in the Table. The clinical diagnosis of BVMD was based on fundus appearance, family history, and a decreased Arden ratio on EOG. The latter test was performed according to International Society for Clinical Electrophysiology of Vision (ISCEV) standards.28 Best vitelliform macular dystrophy patients were staged based on fundus appearance, family history, and a decreased Arden ratio on EOG. The latter test was performed according to International Society for Clinical Electrophysiology of Vision (ISCEV) standards.28
13.6 years, range, 5–60 years) reported previously\(^{30}\) (two-sample Kolmogorov-Smirnov test, \(P = 0.28\)); qAF values obtained from the latter subjects served as control data of the study. Other information about the study cohort can be found in the Table. The BVMD eyes were significantly more hyperopic than those of healthy controls (mixed-effects regression, \(P < 0.001\)). Similarly, the axial lengths of BVMD eyes were shorter than for control eyes (mixed-effects regression, \(P = 0.001\)). Among the BVMD patients there was no difference in axial length (\(P = 0.59\)) and refraction (\(P = 0.93\)) between left and right eyes (two-sample Kolmogorov-Smirnov test).

All procedures adhered to the tenets of the Declaration of Helsinki, and written informed consent was obtained from all patients after a full explanation of the procedures was provided. The protocol was approved by the institutional review board of Columbia University (New York, NY).

### Image Acquisition

Autofluorescent images (30\(^{\circ}\); 488-nm excitation) were acquired using a cSLO (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany) modified by the insertion of an internal fluorescent reference to account for variable laser power and detector gain.\(^{27}\) The barrier filter in the device transmitted light from 500 to 680 nm. Before image acquisition, pupils were dilated to at least 7 mm with topical 1% tropicamide and 2.5% phenylephrine. With room lights turned off, a near-infrared reflectance (NIR-R) image was recorded first. After switching to AF mode (488-nm excitation; beam power < 260 \(\mu\)W) the camera was slowly moved toward the patient to allow the patient to adapt to the blue light. Patients were asked to focus on the central fixation light of the device. The fundus was exposed for 20 to 30 seconds to bleach rhodopsin,\(^{27}\) while the focus and alignment were refined to produce maximum and uniform signal over the whole field. The detector sensitivity was adjusted so that the GL did not exceed the linear range of the detector (GL < 175).\(^{27}\) Two or more images were then recorded (each of nine frames, in video format) in the high-speed mode (8.9 frames/s) within a 30\(^{\circ}\) field (768 \(\times\) 768 pixels). To assess repeatability, a second session of AF images was recorded in a subset of eyes (10 eyes of six patients) after repositioning patient and camera. After imaging, all videos were inspected for image quality and consistency in GLs by two of the authors (TD, JPG). For an imaging session, two videos were selected to generate the AF images for analysis. Only frames without localized or generalized decreased AF signal (due to eyelid interference or iris obstruction) and no large misalignment of frames (causing double images after alignment) were considered. The frames then were aligned and averaged with the system software and saved in nonnormalized mode (no histogram stretching).

In addition, a horizontal 9-nm SD-OCT scan through the fovea was acquired in high-resolution mode as an average of 100 individual scans. Optical depth resolution in the Spectralis is approximately 7 \(\mu\)m. Color images were obtained with the FF450+IR fundus camera (Carl Zeiss Meditec, Jena, Germany). Images of the same field were registered to each other using custom software\(^{31}\) running in Matlab (Math-Works, Natick, MA).

### AF Image Analysis

Autofluorescence images were analyzed under the control of an experienced operator with dedicated image analysis software written in IGOR (WaveMetrics, Lake Oswego, OR).\(^{27}\) The software recorded the mean GLs of the internal reference and of three rings (outer, middle, and inner), each consisting of eight circularly arranged segments (Fig. 1), and a software algorithm accounted for the presence of vessels in the sampling area.\(^{27}\) Spectral-domain OCT scans (registered to AF or NIR-R images) were used to estimate the position of the fovea in patients with a macular lesion. The GLs of each segment were calibrated to the reference, zero GL, magnification, and optical media density from normative data on lens transmission spectra\(^{32}\) to give qAF.\(^{27}\) Magnification is determined by the cSLO optics and the refraction status of the eye; the significant difference in refraction of the BVMD eyes compared with that of healthy eyes was thus accounted for.

We used the outer contour of the area exhibiting a high AF signal to define the limits of the macular lesion in AF images.
For each eye nonlesion mean qAF values were obtained for the middle ring and for the inner ring (Fig. 1). To determine the mean qAF outside the lesion, only segments of the middle and inner ring that did not overlap with the lesion were included in the analysis. To this end, for the middle ring, in eight eyes (six patients) it was necessary to exclude one to three segments that overlapped with high AF lesion areas. For the inner ring, in three eyes (three patients) one to three segments had to be excluded. Inner ring measurements were not included for 11 eyes (eight patients) in which more than three segments overlapped with the high AF lesion (Fig. 1B). From all remaining segments of a ring the mean qAF (nonlesion retina) was generated. By taking topographic AF variations (the superotemporal fundus generally has the highest AF signal) and known instrument nonuniformities into consideration, we estimated that exclusion of segments would affect mean qAF by less than $\pm 2\%$. Mean qAF values were based on two images; when a second session of AF imaging had been performed the averages were based on four images. Subsequently, comparison was made to values from previously published healthy controls (277 subjects). We used the Bland-Altman method to test the between-session repeatability of nonlesion qAF measurements obtained from the middle ring in 10 eyes of six patients, and to assess the agreement of qAF measurements obtained from the middle ring between left and right eyes in 11 patients.

The maximum qAF in a $26 \times 26$ pixel area was automatically measured in IGOR (WaveMetrics) for patients in the vitelliform and pseudohypopyon stages. Measurements from all available images were averaged for each eye.

### Spectrofluorometry

In addition to the patients described in the Table, in three patients (ages, 9–46 years), having a clinical diagnosis of BVMD, in vivo spectrofluorometric measurements were obtained within the vitelliform lesion. As previously described, emissions were recorded between 520 and 740 nm with 470-nm excitation (20-nm bandwidth). The data were corrected for absorption by the ocular media. The protocol for these measurements was approved by the institutional review board of the Schepens Eye Research Institute (Harvard Medical School, MA).

### SD-OCT Segmentation

Horizontal SD-OCT line scans through the fovea from one eye per patient were manually segmented, aided by a custom computer program operating in Matlab (MathWorks). The segmentation technique has been described previously and was shown to have good reliability. For each patient, the eye with the smaller lesion size was analyzed. If no lesion was present, the right eye was chosen for analysis. Eight borders, labeled 1 through 8 in Figure 2, were segmented: (1) the border between vitreous and inner limiting membrane (ILM), (2) the border between retinal nerve fiber layer (RNFL) and retinal ganglion cell layer (RGC), (3) the border between inner plexiform layer (IPL) and inner nuclear layer (INL), (4) the border between INL and outer plexiform layer (OPL), (5) the center of the outer limiting membrane (OLM), (6) the center of the hyperreflective inner segment ellipsoid (ISE) band, (7) the proximal border of the retinal pigment epithelium (pRPE), and (8) the border between Bruch’s membrane (BM) and choroid. As shown in Figure 2, six retinal layers were derived from these eight boundaries: a. RNFL: distance between ILM (1) and RNFL/RGC (2); b. RGC+: distance between RNFL/RGC (2) and IPL/INL (3); c. INL: distance between IPL/INL (3) and INL/OPL (4); d. OPL: distance between INL/OPL (4) and OLM (5); e. OS (outer segment): distance between ISE (6) and pRPE (7); and f. RPE+: distance between pRPE (7) and BM/choroid (8). Mean thicknesses for each of the six layers were compared with those of 35 healthy subjects (mean age 45.1 ± 15.0 years, range, 15–65 years).
Statistical Analyses

Analyses were performed using Prism 5 (GraphPad Software, La Jolla, CA) and Stata 12.1 (Stata Corp., College Station, TX). Where appropriate, we used mixed-effects linear regression that accounts for within-subject correlations between eyes and between close family members (e.g., sibling, parent–child).

RESULTS

Multimodal Imaging

Vitelliform lesions of BVMD have typically been defined by their appearance on color fundus photographs. We used the outer contour of the area exhibiting high AF signal (dashed vertical lines in Figs. 3A–6A) to define the limits of the macular lesion in AF images. With image registration, this boundary exhibited good correspondence to the lesion observed in color fundus photographs (Figs. 3C–5C) and to the altered reflectance observed in NIR-R (Figs. 3B–6B). In one eye in the vitelliruptive stage the lesion exhibited reduced AF signal throughout. To define the limits of the macular lesion in this case, we relied on the outer border of the area of reduced AF.

On SD-OCT the vitelliform lesions were typically dome-shaped. Comparison of the AF and SD-OCT images indicated that the high AF signal originated from an area of separation between the hyperreflective bands attributable to the photoreceptor inner segment ellipsoid (ISe; previously referred to as inner segment/outer segment (IS/OS) junction), (3) eOS, and (4) RPE/BM complex. Assignment of reflectivity bands is based on Spaide and Curcio. In the vitelliform stage (Fig. 3), the ISe band was intact but displaced toward the inner retina. In the pseudohypopyon stage (Fig. 4), the ISe and eOS bands were interrupted within the lesion and the eOS band was thickened at the margin of the lesion. By the vitelliruptive stage (Figs. 5–7), the lesion was predominantly hyporeflective centrally and the AF signal was also diminished. Stalactite-like reflective material extended into the lesion from above, and mounds of reflective material were present at the RPE/BM surface. However, in some patients localized spots of high AF were present at the lesion border; these spots corresponded to hyperreflective material in SD-OCT images (Fig. 7).

We noticed a low AF halo in two patients, P16 (right eye) and P5 (left eye; illustrated in Fig. 6); this halo exhibited a well-defined border on NIR-R and resembled a blister. This low AF halo surrounded the high AF lesion area and correlated with thinning of the outer nuclear layer (ONL) on SD-OCT. Without corroborating NIR-R imaging, the low AF halo in AF images may be difficult to distinguish from the normal distribution of macular pigment and/or the area of higher RPE melanin.

Quantitative Fundus Autofluorescence (qAF)

Quantitative AF images were obtained from 27 eyes of the 16 BVMD patients. Quantitative AF was measured in two rings (middle and inner), each consisting of eight circularly arranged segments (Fig. 1). Segments that did not overlap with the high AF lesion were used for nonlesion measurements. In all BVMD eyes, nonlesion qAF was within normal limits (95% confidence
interval (CI) for age and race/ethnicity (Fig. 8). Among the BVMD patients, qAF increased with age (mixed-effects regression, \( P < 0.001 \)); this age-related increase was the same for BVMD and healthy eyes \( (P = 0.7) \), and also when corrected for race/ethnicity \( (P = 0.6) \). There was no difference in the nonlesion qAF between eyes in the subclinical stage and later disease stages when corrected for age \( (P = 0.4) \).

Maximum lesion qAF levels (mean ± SD) measured in a 26 × 26 pixel area, were 557 ± 5 and 760 ± 3 qAF units, respectively, for the right and left eye of P7 (age: 5 years, pseudohypopyon stage); 274 ± 9 and 329 ± 10 qAF units, respectively, for the right and left eye of P10 (age: 33 years, central lesion of multifocal vitelliform); and 113 ± 4 qAF units for the left eye of P14 (age: 14 years, vitelliform stage). These levels were more than 23 (P7, \( P < 0.001 \)), 2.2 (P10, \( P = 0.04 \)), and 1.4 (P14, \( P = 0.2 \)) times higher than the mean qAF inside the inner ring for healthy subjects of the same age and race/ethnicity (data not shown).

For nonlesion qAF measured in the middle ring of right and left eyes, the Bland-Altman coefficient of agreement was 19.2%.

We measured the between-session Bland-Altman coefficient of repeatability in 10 eyes of 6 patients. The intersession coefficient of repeatability was \( 6.7.0\% \) (95% CI).

**Spectrofluorometry**

The shapes of the emission spectra recorded within the vitelliform lesions of BVMD patients were consistent with emission spectra of lipofuscin recorded spectrofluorometrically in healthy eyes (Fig. 9). In all cases emission maxima were at 580 to 620 nm.

**SD-OCT Segmentation**

Figure 10 shows the thickness profiles (mean and 95% CI) of BVMD patients with a lesion (red profiles) compared with healthy controls (black profiles). The dashed vertical lines indicate the average position of the lesion across all BVMD patients; the latter position was defined by AF (as described in the Multimodal Imaging section). Abnormalities in retinal thickness were limited to the outer retina; inner retinal layers (RNFL, RGC+, and INL) were within normal limits throughout the retina. The ONL thickness was decreased in the lesion area and appeared to be increased outside the lesion border. The OS layer was thickened within the lesion. This thickening was due, at least in part, to the subretinal fluid and extended outside the lesion border. Based on the cohort average, the RPE+ thickness was within normal limits throughout the retina.

Figure 11 shows the individual thickness profiles of six subclinical BVMD patients, which were within the 95% CI (thin black lines) of the controls.

**DISCUSSION**

Quantitative fundus AF in BVMD revealed that fundus areas outside the central lesion do not exhibit increased lipofuscin levels. Within the fluid-filled lesion, qAF levels were elevated compared with corresponding fundus areas in age-similar controls. In our cohort, the patient in the pseudohypopyon stage exhibited higher lesion qAF levels than the two patients in the vitelliform stage. Emission spectra recorded at the fundus in BVMD patients exhibited maxima at 580 to 620 nm, these maxima are similar to that recorded in healthy eyes (Fig.
Figure 5. Vitelliruptive stage (P1). With image registration, corresponding positions in the images (A–D) are shown as dashed vertical lines. (A) Fundus AF image. The center of the lesion appears mottled and of low AF and is surrounded by a thin ring of higher AF with intermittent small bright puncta (outer border indicated by dashed line). (B) Near-infrared reflectance emphasizes the contours of the lesion. (C) Color image. Multiple aggregates of yellowish material are visible in the central part of the lesion. These aggregates do not necessarily emit a high AF signal (compare with panel A). (D) Spectral-domain OCT. Horizontal axis and extent of the scan are indicated by green arrow in (A). The dome-shaped predominantly hyporeflective lesion is situated between reflective bands attributable to OLM and RPE/BM. The lesion interrupts the bands attributable to the ISe and eOS. Within the lesion, stalactite-like reflective material extends from above and mounds of reflective material settle below. The ONL thickness is severely reduced over the lesion. In the area that correlates with the higher AF ring, the eOS band is dissolving, and attenuated in intensity and appears thickened adjacent to the lesion. White arrows indicate area of increased choroidal reflectivity; this phenomenon is generally interpreted as a sign of RPE atrophy.

Figure 6. Vitelliruptive stage (P3). With image registration, corresponding positions in the images (A–C) are shown as dotted and dashed vertical lines. (A) Fundus AF reveals a round central area with irregularly increased AF and intermittent small bright puncta. This area of increased AF is surrounded by a sharply delineated homogenous low AF halo (outer border indicated by white arrowheads). (B) On NIR-R a three-dimensional appearing circular blister with sharply defined borders is visible. The blister surrounds the vitelliruptive lesion and corresponds to the low AF halo in (A) (white arrowheads). (C) Spectral-domain OCT. Axis and horizontal extent of the scan are indicated by green arrow in (A). Spectral-domain OCT reveals a dome-shaped predominantly hyporeflective lesion located between the reflective bands attributable to OLM and RPE/BM. The ISe and eOS bands are interrupted. The ONL is thinned within the area of the blister-like lesion (between dotted and dashed lines).
abnormal multifocal electroretinograms (mfERGs) correspond precisely to the area of the lesion. Recent findings from adaptive optics scanning laser ophthalmoscopy have also indicated that photoreceptor cells are normal in areas immediately adjacent to clinically visible lesion. Thus, an abnormality of the nonlesion retina is not obvious. Optical coherence tomography studies of BVMD patients have yielded differing viewpoints on the intactness of the RPE/Bruch’s membrane. Our segmentation data indicated that in the case of our cohort RPE/Bruch’s membrane when averaged was within normal limits, even within the lesion. However, as shown in Figure 5, we cannot exclude RPE thinning in advanced cases. Our segmentation data also indicated that there was an apparent thickening of the ONL outside the lesioned area. If real, this would be another example of a thickening of the ONL in a retinal degenerative disease.

Regarding the predilection of BVMD for the macula, it has been reported that immunohistochemical staining together with quantitative PCR and immunoblotting indicated that bestrophin-1 expression was more robust in the peripheral retina than in the macula. Unique environmental stresses imposed upon macular RPE have been proposed as an alternative explanation. Interestingly, in canines, several coding changes in the canine BEST1 are associated with a recessive disease featuring multifocal areas of fluid-filled retinal elevations similar to human BVMD.

The light peak (LP) of the EOG is generated by a Ca\(^{2+}\)-dependent outward Cl\(^{−}\) conductance across the basal membrane of RPE. Bestrophin-1 is a membrane protein expressed basolaterally in the RPE, but a large proportion of the protein is found in association with cytosolic membrane where it may serve as an anion channel. Most investigations have shown that mutations in bestrophin-1 result in a loss of anion channel conductance. Nevertheless, the role of BEST1 in mediating the LP has been difficult to elucidate and mouse models have not faithfully replicated BEST1-related disease in humans. Several channel functions have been assigned to bestrophin-1 (Ca\(^{2+}\)-activated Cl\(^{−}\), Ca\(^{2+}\) channel regulator, volume regulated Cl\(^{−}\), and HCO\(_3\)^{−}). Because of osmotic forces, the efflux of both Cl\(^{−}\) and HCO\(_3\)^{−} across the basolateral membrane of RPE is followed by fluid transport from the subretinal space to the choroid. Thus, failure of fluid transport in the presence of a BEST1 mutation is likely the cause of the fluid-filled neural retinal detachment. Accordingly, disrupted fluid flux has been observed in a human-induced
The ionic milieu of photoreceptor cell outer segments is also presumably altered in BVMD, resulting in not only an accumulation of fluid but also outer segment debris. The retinal detachment created by the accumulation of fluid likely interferes with the phagocytosis of shed outer segment membranes, which therefore accumulate subretinally. Thus, much or all of the vitelliform material probably originates from unphagocytosed outer segment membrane in the subretinal fluid responsible for the detachment. Since the bisretinoid fluorophores of RPE lipofuscin originate from outer segments, these bisretinoids and bisretinoid precursors in the outer segment could be the source of the AF. The spectrofluorometric data presented here are consistent with these views. Even then however, the fluorescence is particularly bright, much brighter than the lipofuscin in surrounding RPE. Thus, in addition to impeded removal of outer segment debris, the possibility remains that the rate of bisretinoid formation in the photoreceptor outer segments is accelerated. Eventual loss of the fluorescence associated with the lesion could involve clearance of the subretinal photoreceptor cell debris perhaps by macrophages, in addition to photodegradation of the fluorophores. It is perhaps the yellow color and autofluorescence of the vitelliform material in combination with uncertainty as to the structural correlates of the lesion that lead to the assumption that RPE lipofuscin accumulation is increased throughout the fundus in BVMD. Based on our findings, we suggest that increased RPE lipofuscin is not a feature of the primary disease process; however, the increased AF levels originating from pluripotent stem cell model of BVMD. 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Hypermia and short axial length are characteristic findings in autosomal recessive bestrophinopathy (ARB). In which patients by definition carry a BEST1 mutation on both chromosomes,22 and it has been suggested that functional bestrophin-1 may be required for the development of the eye.3,61,62 The cohort in this study was also hyperopic and had a short axial length (Table). These phenotypical features may therefore be characteristic not only of ARB but also of autosomal dominant BVMD. Previous studies have reported short axial length and a subsequently increased risk for angle-closure glaucoma in three BVMD families.63,64

Patient 10 had a clinical diagnosis of multifocal BVMD, but no mutation in BEST1 was found and there was no clear evidence of a positive family history of BVMD. We can therefore not exclude the possibility that this patient may have been misdiagnosed or represent a phenocopy of BVMD.65

In summary, qAF revealed that mutations in BEST1 do not cause increased lipofuscin levels in nonlesion fundus areas. Spectral-domain OCT segmentation demonstrated characteristic structural abnormalities within the lesion and at a transition zone outside the lesion. Subclinical cases displayed no obvious changes. These findings inform our understanding of the pathogenesis and clinical findings of BVMD.

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