

Diffuse Outer Layer Opacification: A Novel Finding in Patients With Autosomal Recessive Bestrophinopathy

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Purpose: Autosomal recessive bestrophinopathy (ARB) is a rare inherited retinal dystrophy resulted from mutations in bestrophin-1 (*BEST1*) which affect functioning of the retinal pigment epithelium (RPE). Descriptions of disease findings in patients with ARB to date have focused only on macular changes. In this case series, we report previously undescribed mid-peripheral retinal changes occurring in 4 patients with ARB.

Design: Case series.

Methods: A single-center, retrospective review of medical records from Mayo Clinic patients with ARB was performed. Imaging reviewed include fundus photography, fundus autofluorescence, spectral domain optical coherence tomography (OCT), and fluorescein angiography. Demographic information and disease progression were noted.

Results: 4 affected patients from 3 families were identified. All 4 patients were female, and mean age was 12.5 years (range 5–19 years). Diffuse mid-peripheral whitening was consistently noted on fundus photography. Concomitant OCT imaging demonstrated areas of hyperreflectivity in the photoreceptor outer segment layer in areas corresponding to whitening seen on fundus photography. In 1 patient who was followed for 12 years, this finding persisted. Subretinal fluid was also consistently present. Other pathologic imaging findings observed in each patient were in agreement with previous reports of ARB.

Conclusions: This is the first descriptive report of pathologic findings occurred beyond the posterior pole in patients with ARB. These mid-peripheral retinal changes potentially imply that the entirety of the RPE is affected by mutations in *BEST1*, as also suggested by previous electro-oculogram (EOG) findings. Such implications will be important when developing treatment trials, as past trials have focused only on the posterior pole of the RPE.

Key Words: autosomal recessive bestrophinopathy, *BEST1*, retina

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The bestrophinopathies are a spectrum of inherited retinal dystrophies resulted from mutations affecting the *BEST1* gene. *BEST1* encodes a 585 amino acid transmembrane protein, bestrophin-1, which is located in the basolateral membrane of retinal RPE cells.^{1,2} Bestrophin-1 is thought to serve as a

Ca²⁺-activated anion channel and an inhibitor of intracellular Ca²⁺ signaling.^{3–5} Mutations affecting *BEST1* result in impaired phagocytosis of photoreceptor outer segments, leading to aberrant rod and cone function.^{5,6} The best characterized bestrophinopathy is Best vitelliform macular dystrophy (or Best disease). Other variants include adult onset vitelliform macular dystrophy, autosomal dominant vitreoretinopathy, retinitis pigmentosa 50 (RP50), and ARB. A recent study conducted by our group had found that the prevalence of Best disease in the United States is on the order of 1 in 16,500 to 1 in 21,000 individuals.⁷

First characterized in detail in 2008, ARB is differentiated from the other bestrophinopathies primarily by its inheritance pattern, and its juvenile age of onset of disease signs and symptoms.^{8,9} Both compound heterozygous and homozygous *BEST1* gene mutations have been reported to cause ARB.⁹ Defective phagocytosis of the photoreceptor outer segments by the RPE is thought to lead to the marked photoreceptor abnormalities seen in ARB that are reflected by changes including thickening and elongation (or “stalactites”).^{10,11} This faulty phagocytosis may be due in part to release of large amounts of osmolytes before shedding the photoreceptor outer segments, adversely influencing the role of bestrophin in RPE cell membranes.¹² Other classic findings that have been consistently described include subretinal scarring, small vitelliform lesions surrounding the macula that may fluctuate and diminish with time, small yellowish subretinal deposits, and subretinal fluid and cystoid macular changes.¹³ Given its aberrant anion channel function, EOG generally demonstrates reduced light peak-to-dark trough ratio (LP:DT, or Arden ratio), reflecting a diffuse diminished ability of the RPE to depolarize.^{14–16} Similarly, full-field electroretinogram (ERG) often demonstrates abnormalities in cone and rod responses, including prolonged latencies and reduced amplitudes.^{17,18} Of note, it has been reported that in young children with ARB the full-field ERG may be normal, whereas in older children the full-field ERG may become abnormal.¹⁹ Genetic testing is essential for diagnosis of ARB and other bestrophinopathies, as they may otherwise be difficult to be differentiated from similarly presenting vitelliform diseases.⁸

There are currently no therapies available for ARB or definitive treatment for any of the bestrophinopathies. Management of ARB is mainly symptomatic, and focuses on prevention and attenuation of vision-threatening complications. Treatment of amblyopia and surgical strabismus correction are often required, and prophylactic laser peripheral iridotomy may be performed to prevent angle closure and treat glaucoma. Anti-vascular endothelial growth factor (anti-VEGF) treatment has been used with success in some patients for treatment of the choroidal neovascularization (CNV) that can occur early in the disease course.²⁰ The use of iPSC-derived RPE cells in studying mutations underlying ARB has helped enhance understanding of

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disease pathogenesis.¹³ Continued investigation will be necessary to determine definitive genotype–phenotype correlations in patients with ARB. It is noteworthy that imaging changes in the literature to date have focused primarily on pathologic changes occurring in the macula, and few observations have been consistently reported beyond this area. Limited extramacular observations in some patients with ARB have included hyperautofluorescent deposits corresponding with extramacular multifocal yellowish lesions.^{19,21} However, we are demonstrating 4 patients with varying phenotypic severity of ARB who all have similar opacification of the mid-periphery on fundus photography. Although the findings might look like a prominent retinal nerve fiber layer (RNFL) on fundus photography, which may be observed in a normal pediatric fundus, OCT shows hyperreflectivity in the photoreceptor layer in these areas. These findings help show that multimodal imaging can help demonstrate changes of the retina outside the macular region, which we have termed diffuse outer layer opacification (DOLO), to bring attention to the fact that the RPE outside the macular area may be affected in ARB. As trials are developing in pursuit of a treatment for this disease, it will be necessary to take the entirety of the retina into consideration but not just posterior pole RPE abnormalities.

METHODS

A retrospective, single-center observational case series was performed. The procedure used in this study adhered to the tenets of the Declaration of Helsinki and was approved by the Mayo Clinic Institutional Review Board. We reviewed the electronic medical records of 4 patients from 3 unrelated families who were evaluated in the outpatient setting at Mayo Clinic in Rochester, Minnesota with diagnosis of ARB. The diagnosis had been confirmed in each patient via genetic testing positive for known pathogenic variants of the *BEST1* gene. Ophthalmologic examinations that were evaluated in each patient's medical record include measurements of best-corrected visual acuity (BCVA), refractive error, ophthalmoscopy, slit lamp biomicroscopy, fundus photography, OCT, fundus autofluorescence (FAF) imaging, fluorescein angiography (FA), and EOG.

RESULTS

All 4 patients in this series were females who were diagnosed with ARB before age 18. 1 patient initially presented and was diagnosed with the disease at our institution, whereas the other 3 patients were diagnosed with ARB at other institutions prior to presenting to us.

Family 1, Patient 1

Patient 1 initially presented to our institution at age 7 with decreased vision owing to bilateral lesions presumed to be secondary to multifocal choroiditis, and subretinal fibrosis of the left eye. BCVA at the time was 20/100 in the right eye (OD) and 20/50 in the left eye (OS), with no improvement on pinhole, and manifest refraction was +5.75 OD and +5.50 OS. Slit lamp biomicroscopy revealed no abnormalities apart from 1–2+ cells in the vitreous OD. Fundus evaluation and photographs at the time revealed bilateral subretinal fibrosis, yellowish pigment in the fovea, small white lesions within the borders of the superior and

inferior arcades, and diffuse whitening in the mid-periphery (Fig. 1A, B). OCT imaging showed subretinal fluid, subretinal debris, and elongation of photoreceptor outer segments. Additionally, there were areas of hyperreflectivity on OCT in the photoreceptor OS layer which seemed to correspond with areas of whitening on fundus photography (Fig. 1C, D).

Further workup led to a diagnosis of ARB after gene analysis, whereby the patient was found to have compound heterozygous *BEST1* mutations Arg141His (CGC > CAC) and I366fsX18 (del10atCAGGTGTGGC). Family history was negative for ophthalmic disease, but further testing revealed each parent to be a heterozygous carrier for one of the mutations. The patient has been routinely followed at our clinic since presentation, most recently within the past several months. OCT images have consistently continued to demonstrate subretinal fluid, subretinal debris, and elongation of photoreceptor outer segments, although the amount of subretinal fluid has subtly increased from 2012 to 2019. OCT continues to demonstrate hyperreflectivity of the photoreceptor outer segments in areas corresponding to the mid-peripheral whitening seen on fundus photography (Fig. 1E–H). Fundus imaging has remained remarkably similar over time. The patient's BCVA has remained stable during the last several years at 20/20 OD and 20/50 OS, with dense central scotomata on automated visual fields bilaterally consistent with macular disease.

Family 2, Patient 2

In stark contrast, the other 3 patients were referrals from outside institutions with workup from ARB already completed. Patient 2 presented at age 5 with genetic testing significant for 2 heterozygous pathogenic variants, 1 being c.636 + 1G > A, IVS5 + 1G > A, and 1 being c.604 C > T, p.R202W. The patient's BCVA was 20/50 + 2 OD and 20/25 + 1 OS, with a corrected refraction of +2.50 and +2.00. Slit lamp biomicroscopy was unrevealing. EOG demonstrated marked reduction of the Arden ratio to 1 bilaterally. IOP was within normal limits. Funduscopic examination on intake revealed a single foveal vitelliform lesion in the right eye and multifocal vitelliform lesions in the left eye, and on fundus photography mild diffuse opacification was noted to be present mid-peripherally in both eyes (Fig. 2A, B). OCT demonstrated a large central scar in the right eye with extensive detachment, and vitelliform lesions, subretinal fluid, and intraretinal cysts bilaterally. In addition, hyperreflectivity of the photoreceptor outer segments was apparent in areas corresponding to the diffuse whitening seen on fundus photography (Fig. 2C, D). The patient has continued to be followed at our clinic and imaging has remained stable, despite a trial of dorzolamide for the intraretinal fluid. At a recent 3-year follow-up visit, imaging showed consistent findings of DOLO bilaterally on both fundus photography and FAF (Fig. 2E, F).

Family 3, Patients 3 and 4

Patients 3 and 4 were biological siblings who initially presented to our institution together at ages 19 and 16, respectively. Both were confirmed to have ARB with the same heterozygous pathogenic variants of the *BEST1* gene, Glu35Lys (GAG > AAG) and Arg195Val (GCG > GTG). Patient 3 had a several years of history of stable flashes, floaters and occasional eye pain, although BCVA was 20/20-1 OD and 20/30-2 OS, with manifest refraction of +1.25 OD and +0.50 OS. She had

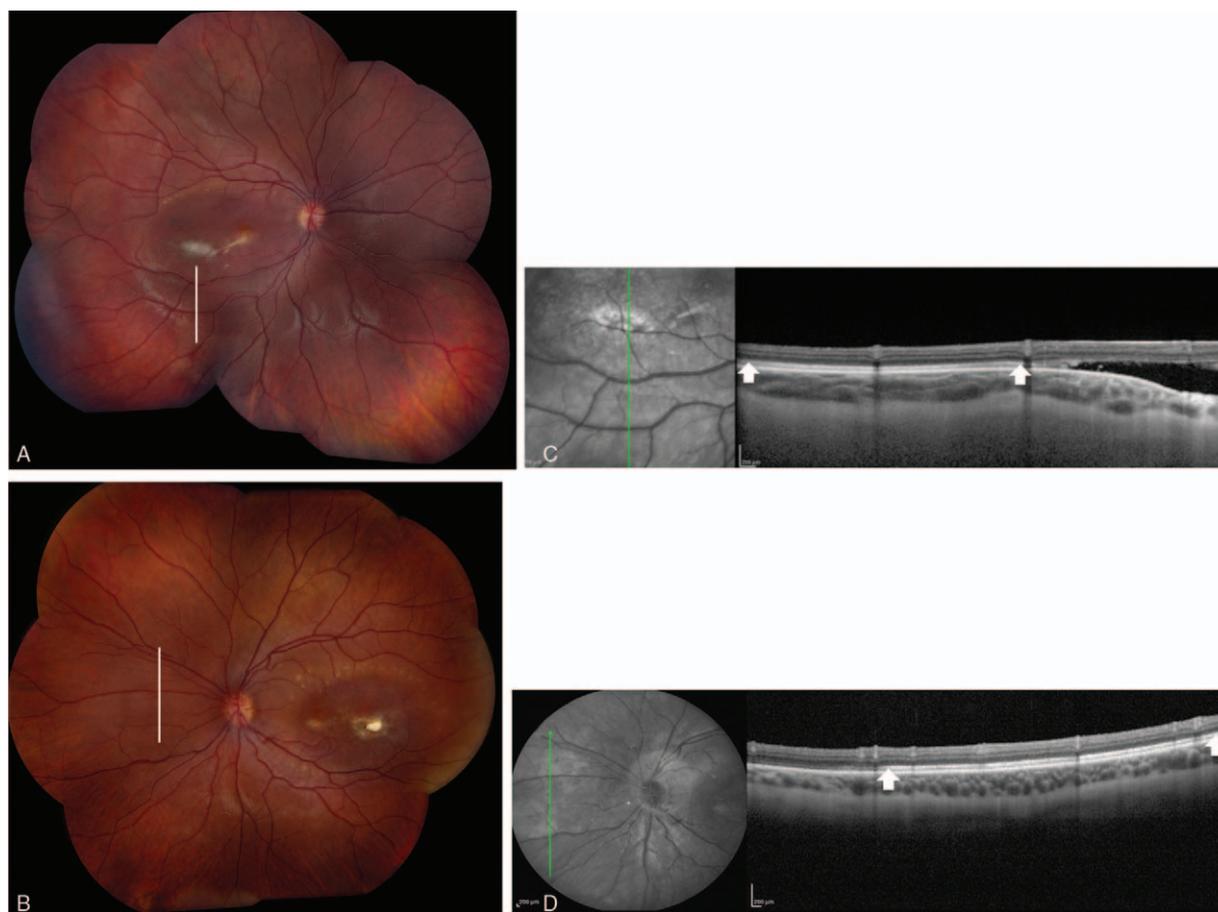


Figure 1. Patient #1 was diagnosed with ARB at our clinic at age 7. Fundus photography of her right eye at the time of diagnosis (A) demonstrated subretinal fibrosis and small vitelliform lesions scattered throughout the arcades. OCT imaging shows concomitant hyperreflectance in the ellipsoid zone and photoreceptor outer segment (OS) layer, as bounded laterally by the white arrows (C). These findings were present bilaterally and have remained unchanged during 12 years of follow-up, as represented in similar imaging of the left eye at recent visit (B and D). ARB indicates autosomal recessive bestrophinopathy; OCT, optical coherence tomography.

undergone 3 rounds of photodynamic laser treatment 6 year before, with no further interventions since that time. Slit lamp biomicroscopy was unrevealing apart from some primary acquired melanosis of the limbus OD. Funduscopic examination showed multiple yellowish spots along and within the arcades OD, and some scarring at the center and superotemporal to the fovea. Fundus photos demonstrated diffuse whitening along the inferior arcades bilaterally (Fig. 3A, B) and in the superotemporal periphery in the right eye (not shown). These areas had the same distinct pattern of corresponding outer segment hyperreflectivity on concomitant OCT imaging (Fig. 3C, D). Fluorescein angiography (FA) revealed early window deficits as evidenced by hyperfluorescence scattered throughout the macula. In addition, cystoid macular edema was present in the left eye on OCT, and both eyes showed a blunted foveal contour, subretinal fluid throughout the macula, intraretinal fluid superior and inferior to fovea, and a shaggy photoreceptor layer.

In contrast, Patient 4 had less severe visual manifestations, and complained about a 2-year experience of slight worsening vision that fluctuated with stress and lack of sleep. BCVA was 20/30-1 OD and 20/30+1 OS. Refraction was not obtained at the initial visit. Slit lamp biomicroscopy was unrevealing. Funduscopic examination revealed yellowish accumulations bilaterally, subretinal fluid superior to the disc on the right and

superotemporally to the fovea on the left, and subretinal heme just outside the arcade on the left. A small white pigment clump was present inferiorly in the mid-periphery. Mid-peripheral whitening was notable superotemporally in both eyes on wide-field composite OCT imaging (Fig. 4A, B). FA showed prominent subretinal deposits surrounding the macula bilaterally. OCT was almost unremarkable in comparison to that of the patient's sibling, apart from an area of cystoid macular edema in the left eye and hyperreflectivity of the outer segment layer in an area corresponding to the mid-peripheral whitening as seen in Patients 1 to 3 (Fig. 4C, D).

DISCUSSION

In this case series, we presented a description of mid-peripheral whitening found to be consistently present in 4 patients with ARB. We have defined the DOLO observed in these patients as mid-peripheral whitening observed with both fundus photography and FAF which distinctly corresponds with hyperreflectivity of the photoreceptor outer segment layer on concomitant OCT imaging. The hyperreflectivity noted was distinctly separated from areas of subretinal fluid, refuting the possibility that this whitening could be occurring as a result of fluid accumulation. Moreover, these areas of whitening were seen on funduscopy in

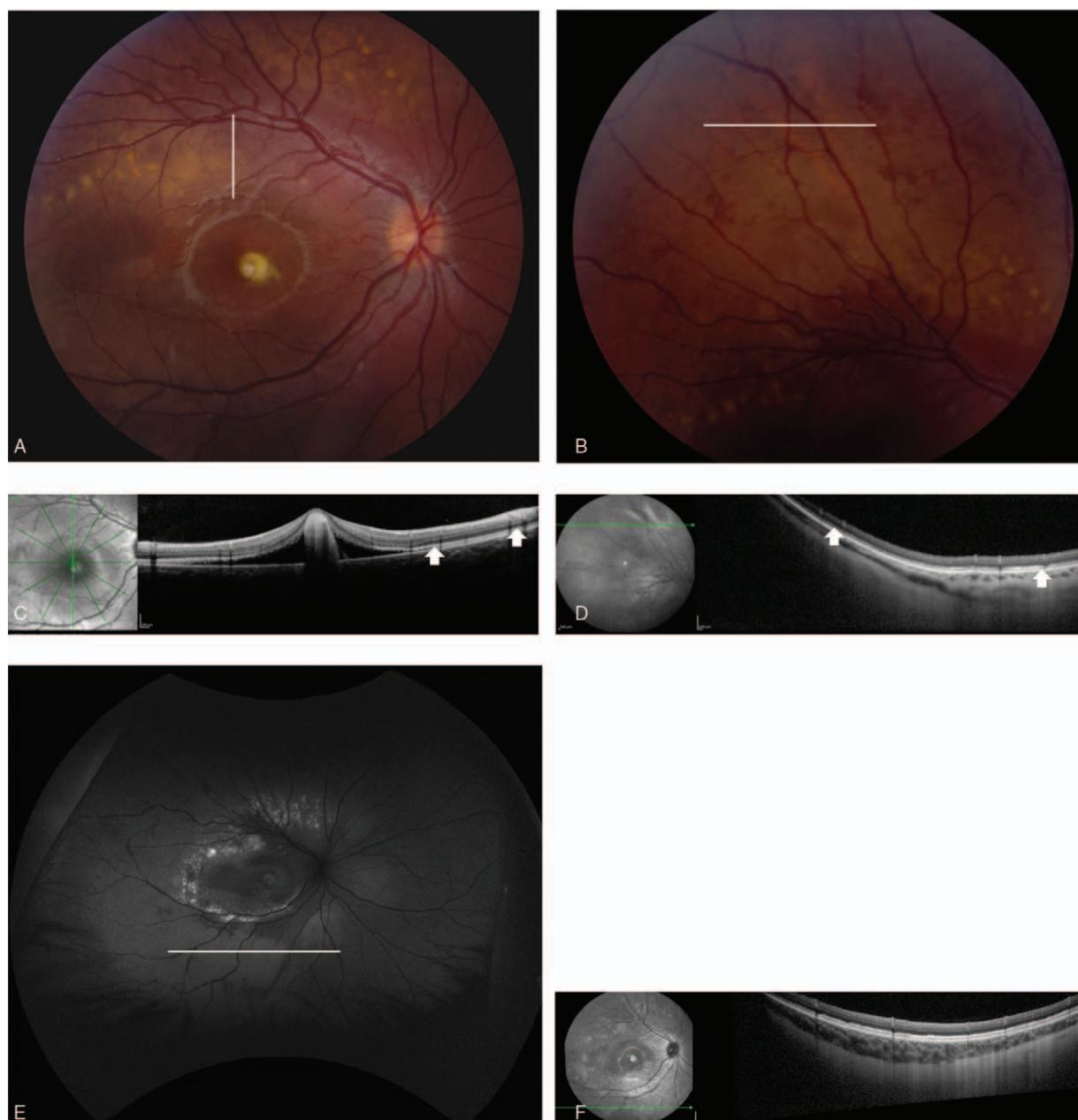


Figure 2. Patient #2 was referred to our clinic at age 5 with ARB. Fundus photographs of the right macula (A) and left temporal periphery (B) are shown. OCT of the right eye (C) shows a large central scar and extensive subretinal detachment, but our attention is instead on hyperreflectiveness of the ellipsoid zone and photoreceptor outer segment (OS) layer (bounded by arrows) which corresponds distinctly to areas of whitening in 2A (white bar). The same pattern is noted far peripherally in her left eye as shown in (B) and (D). Imaging obtained at a return visit 3 years later shows FAF outside the macular area (2E). Concomitant OCT imaging through an area of FAF also shows hyperreflection of the EZ and photoreceptor OS layers (F, entirety). ARB indicates autosomal recessive bestrophinopathy; FAF, fundus autofluorescence; OCT, optical coherence tomography.

each patient in addition to fundus photography. The other imaging findings and functional data obtained in these 4 patients were in agreement with previous reports of ARB.

These findings of DOLO in patients with ARB potentially imply that the entirety of the RPE is affected by ARB-associated mutations in the *BEST1* gene, not just the posterior pole. This speculation is supported by prior knowledge that the Arden ratio is diminished in ARB, a commonality in diseases with diffuse RPE involvement. Interestingly, the EOG in patients with ARB tends to be disproportionately abnormal with what can be explained by rod-mediated ERG reduction.¹⁶ Although beyond the scope of this study, further extraction of clinical features of the mutation carriers noted above from previous reports would be an additional

helpful step. This could allow us to determine whether the mid-peripheral retinal changes described here are correlated to specific *BEST1* mutations, such as those specific to our patients.

For comparison to the dominant forms of the bestrophinopathies, previous studies investigating Best vitelliform macular dystrophy have investigated differences in the macular and peripheral RPE in an effort to determine the mechanism by which disease findings present exclusively in the macula. Interestingly, bestrophin expression was found to be higher in the periphery than in the macula.²² This led to speculation that there is loss of function in which the peripheral RPE is able to compensate and function more normally with one wild-type copy than the macular RPE, or by contrast that loss of one functional allele may result in

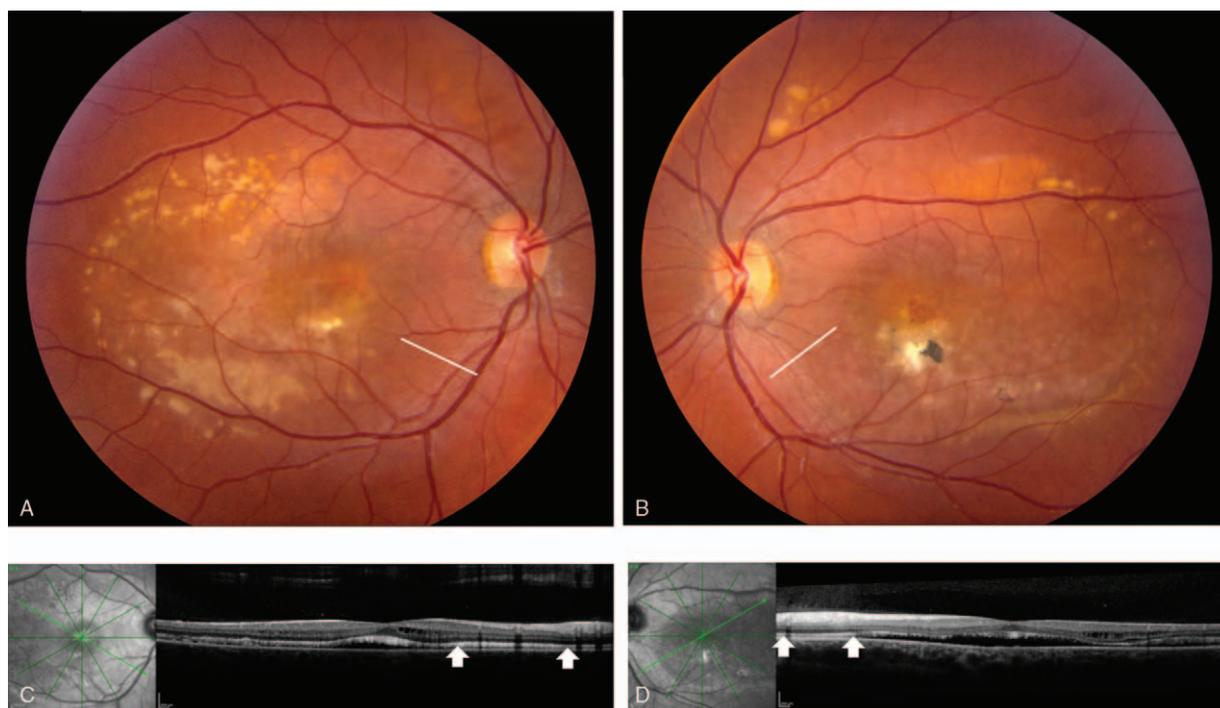


Figure 3. Patient #3 was referred to our clinic at age 13 with a diagnosis of ARB. Bilateral fundus photography with concomitant OCT imaging showed similar findings of DOLO as illustrated before in a similar manner. We focus here on the right inferior arcade (A, C) and left posterior pole (B, D) to demonstrate the fact that this pattern is occurring in the posterior pole and in the mid-periphery, as shown in Patients #1, 2, and 4. ARB indicates autosomal recessive bestrophinopathy; DOLO, diffuse outer layer opacification; OCT, optical coherence tomography.

insufficient bestrophin protein in the macula as a consequence of lower rates of synthesis in this region, with corresponding deficits in ion homeostasis. However, we know from studies on iPSC-derived RPE cells that not all *BEST1* mutations associated with ARB involve loss of anion channel function.¹³

White Without Pressure

Others have suggested that the findings we have described seem to resemble previous reports of white without pressure (WSP). This is a phenomenon in which an area of peripheral retinal whitening blurs the underlying choroid in the absence of

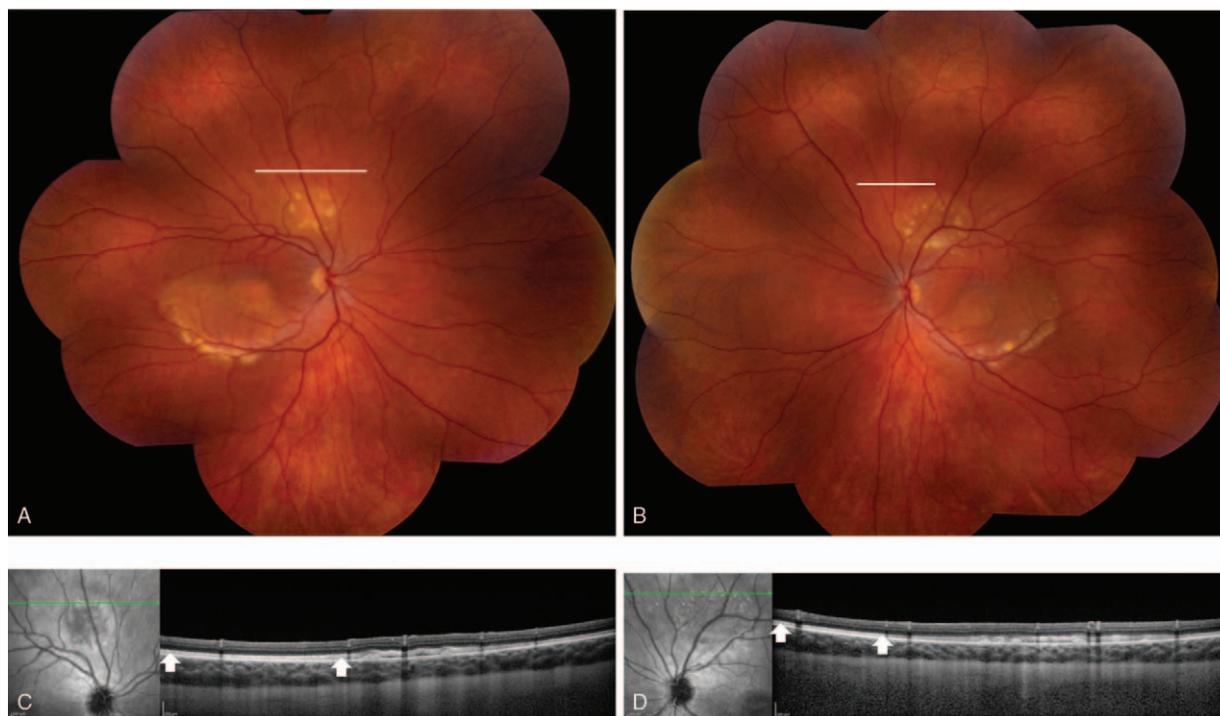


Figure 4. Patient #4 was referred to our clinic at age 16 with a diagnosis of ARB. Bilateral fundus photography (A and B) demonstrated vitelliform lesions superior to the optic disc in both eyes. We again focus here on whitening present in the mid-periphery (white bars) and, in some areas, the far periphery, which corresponds with OS hyperreflectivity on concomitant OCT imaging (areas bounded by arrows in C and D). ARB indicates autosomal recessive bestrophinopathy; OCT, optical coherence tomography.

scleral depression, differentiating it from the normal physiologic finding of WsP that occurs as a result of scleral indentation.²³ Although the cause of WsP is unclear, prevailing speculation into its mechanism involves inward vitreal traction on the peripheral retina. OCT and multimodal imaging studies have localized WsP lesions to the outer retina, as WsP has been shown to correspond to a hyperreflectivity of the ellipsoid zone and interdigitation zone on spectral domain OCT.^{24,25} These findings disprove previous theories of vitreoretinal interface abnormalities. Furthermore, the absence of intraretinal fluid has been confirmed via intraoperative OCT in areas with WsP.²⁶ Although the DOLO which we have described is comparable to WsP in several ways, we note several important distinctions. Visual field testing in patients with WsP lesions does not show a visual defect, and in fact these patients tend to have no decrease in visual acuity. This would suggest that WsP represents a structural as opposed to a functional defect. In contrast, the changes that occur in patients with ARB as a result of *BEST1* mutations ultimately result in a notable decrease in vision. Even at a molecular level, we know that *BEST1* mutations associated with ARB cause objective deficits in bestrophin-1 anion channel function.

Future direction

Attention to the entire RPE will be important as trials are developed in pursuit of a treatment option. Some cellular models have demonstrated functional rescue of ARB-associated mutant bestrophin-1 involving proteasome inhibitors, showing promise for translational research.²⁷ In addition, induced pluripotent stem cell-derived RPE transplantation is currently being studied as a safe therapeutic approach for macular degeneration and related diseases, and could potentially be of great therapeutic benefit in patients with *BEST1* mutations.^{8,28} Investigations behind canine multifocal retinopathy, the orthologous autosomal recessive disease which occurs as a result of mutations in canine *BEST1* (*cBEST1*), have helped develop an important animal model for gene augmentation therapy. Recombinant adeno-associated virus (rAAV) *BEST1* transgene expression in canine multifocal retinopathy-affected retinæ has been shown to be safe and efficacious, showing promise for future development of analogous therapy for human bestrophinopathies.²⁹ As genetic testing becomes more widely adopted, the confirmed prevalence of ARB and other vitelliform dystrophies may encourage further efforts and funding in these areas.⁷ Performing microperimetry and swept-source OCT in patients with ARB may help with better characterization of peripheral retinal disease, and extending these studies to patients with all variations of Best disease may help with further diagnostic assessment and understanding. In addition, now that we have noted this finding in ARB, the other bestrophinopathies should be evaluated for peripheral changes as well.

CONCLUSIONS

This is the first descriptive report of pathologic findings occurring outside of the posterior pole in patients with ARB. The mid-peripheral retinal changes observed on multimodal imaging in these patients imply that the entirety of the RPE is affected by mutations in the *BEST1* gene, as previous EOG findings would also suggest. This may be an important point to keep in mind during the development of treatment trials for ARB. Continued efforts to further characterize gene expression, involved

molecular pathways, and mutations of interest in ARB will help build further our understanding of the pathogenesis of this disease.

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