Differential Macular and Peripheral Expression of Bestrophin in Human Eyes and Its Implication for Best Disease

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PURPOSE. Best disease, or vitelliform macular degeneration, is an autosomal dominant form of macular degeneration that is caused by mutations in the gene encoding bestrophin. On clinical examination, Best disease is characterized by an elevated lesion beneath the neurosensory retina, resembling an egg yolk. The lesions in Best disease are primarily restricted to the macula, a small region of the retina responsible for central vision. The nature of the vitelliform material and the reason the development of such lesions is usually restricted to the macula are two unsolved questions in the pathogenesis of this disorder.

METHODS. The expression of bestrophin protein and mRNA was evaluated by immunohistochemistry, Western blot, and quantitative PCR in a series of normal human eyes. The ultrastructure of the retinal pigment epithelium and the histopathology of two donors with clinically diagnosed Best disease were also examined.

RESULTS. An eye from a Best disease donor with a T6R mutation was found to have deposits containing lipid and glycosconjugates within the central retinal scar. These deposits may be remnants of the vitelliform lesion. Immunohistochemical localization of bestrophin in a series of 22 unaffected eyes revealed a pattern in which macular labeling was less robust than labeling outside the macula in most (18/22) cases. This pattern was confirmed using quantitative PCR and Western blotting.

CONCLUSIONS. Topographic differences in the levels of bestrophin protein may in part explain the propensity for the macula to develop lesions in Best disease. (Invest Ophthalmol Vis Sci. 2007;48:3372–3380) DOI:10.1167/iovs.06-0868

Best disease (juvenile macular dystrophy or vitelliform macular dystrophy) is an autosomal dominant genetic disorder that affects central vision. In its early stages, it is characterized on ophthalmic examination by the presence of an egg yolk–like lesion in the macula. Affected patients also exhibit a diagnostic abnormality detected on the electrooculogram.1–5 The age at which vitelliform lesions develop is highly variable. In some cases, the lesion is likely to be present from birth, whereas in other persons who carry a disease-causing mutation (based on molecular testing or the transmission of the disease phenotype to their offspring), macular findings may develop only with advancing age.4–6 In some patients, this disease results in severe vision loss because the egg yolk lesion can become replaced with an area of gliosis and scarring.7 Best disease may be considered a model system for a more common blinding disease, age-related macular degeneration (AMD), which exhibits similar changes in the retinal pigment epithelium (RPE).

Although Best disease was first described more than 100 years ago,1 relatively little was known about the pathogenesis of this disorder until recently. The gene responsible for Best disease was identified with the use of a positional cloning strategy.7–8 This gene (Best1/VMD2) encodes a protein, designated bestrophin, of 586 amino acids. Bestrophin is expressed predominantly in the RPE.7 Although some details have been disputed, mapping studies generally suggest that bestrophin is a transmembrane protein with four transmembrane domains and with both the amino and carboxyl termini localized within the cytoplasm.10,11 More than 100 distinct mutations in Best1/VMD2 have been described in human eye disease,12 some of which may affect the membrane topology of bestrophin.11 VMD2 mutations do not appear to be associated with AMD.13,14

Identification of the VMD2 gene has led to a number of elegant structural and electrophysiological studies into the pathophysiology of Best disease.15–16 The bestrophin gene is expressed in the basolateral plasma membrane of the RPE in situ and, in specific culture conditions, in vitro.9,17,18 Physiological studies indicate that bestrophin and its related family members most likely serve as calcium-activated/calcium-dependent chloride channels because transfection of cells with the bestrophin gene results in increased chloride conductance.15,19–21 The finding that bestrophin modulates ion flow across the RPE is consistent with the characteristic effect of bestrophin mutations on the electrooculogram, a measure of RPE electrical activity.1,2,22 Additional data suggest that bestrophin may serve to modulate the function of cation calcium channels in the RPE.23,24 Whether bestrophin modulates chloride conductance directly or indirectly is an unresolved question. Biochemical studies of bestrophin purified from porcine eyes indicate that this protein exists in vivo in a dimerized conformation.24 Interestingly, the bestrophin-induced chloride current in transfected cell lines is sensitive to cell volume and therefore may suggest a role for bestrophin in regulating RPE cell volume in vivo.21

Animal models with abnormal bestrophin have also provided new insight into the function of bestrophin and into Best disease. A mouse lacking bestrophin was recently generated by targeted disruption of the bestrophin gene.25 Although mice lacking bestrophin do not appear to develop Best disease–like
lesions or to undergo retinal degeneration, even at 14 months of age, they do exhibit a greatly enhanced intracellular trafficking of calcium after exposure of the RPE to adenosine triphosphate (ATP), consistent with a role in calcium trafficking. In addition to knockout mice, spontaneous mutations in the bestrophin gene of two strains of dogs have been identified that lead to multifocal areas of retinal degeneration in canine multifocal retinopathy (CMR), with lesions histopathologically similar to those in Best disease.

There are a number of intriguing questions about the pathogenesis of Best disease. First, the precise anatomic location and composition of the vitelliform material are uncertain. Histopathologic studies performed to date have not sampled the vitelliform material because few eyes have been studied histologically and all these had undergone macular scarring prior to donation. Optical coherence tomography suggests that the material may lie between the RPE and the photoreceptor outer segments. Second, in most patients with Best disease, the characteristic vitelliform lesion is restricted to the macula, a small area of the posterior pole of the eye delineated by the temporal vascular arcades. Extramacular development of vitelliform lesions is rarely observed, but the predilection for the development of lesions in the macular region in Best disease (and in other macular degenerations) is not well understood. In the present study we describe the histopathologic characteristics of an eye from a donor with a Thr6Arg (T6R) mutation in the bestrophin gene. In this eye, we identified lipid/glycoconjugate-rich material within the retina; although the eyes were obtained after end-stage scarring, it is possible that these deposits represent remnants of the vitelliform lesion. We also evaluated the relative abundance of bestrophin protein and RNA in macular and extramacular regions in a series of normal human eyes and made the surprising observation that the RPE outside of the macula has a relative abundance of bestrophin compared with the macular RPE. This distribution of bestrophin suggests that Best disease results in part from a relative insufficiency of wild-type bestrophin in the macula, and we discuss the significance of this finding in the context of Best disease.

Materials and Methods

Control Human Donor Eyes

Human donor eyes were obtained from the Iowa Lions Eye Bank (Iowa City, IA) and the Nebraska Lions Eye Bank (Omaha, NE). Macular and extramacular punches were collected from nine eyes, as described previously. Superotemporal wedges extending from the pars plana to the scar were also evaluated in 13 eyes. Eyes were fixed in 4% formaldehyde in phosphate-buffered saline (PBS) and were processed as described previously. In addition, 4-mm macular punches centered on the fovea were also evaluated in 13 eyes. Eyes were fixed in 4% formaldehyde in phosphate-buffered saline (PBS) and were processed as described previously. In addition, 4-mm macular punches centered on the fovea were also evaluated in 13 eyes. Eyes were fixed in 4% formaldehyde in phosphate-buffered saline (PBS) and were processed as described previously. 4-mm macular punches were collected from nine normal donor eyes within 7 hours of death and were frozen in liquid nitrogen and stored at -80°C until used for Western blot analysis. After dissection in two additional eyes, 4-mm punches were collected for Western blot analysis from the macula and from each of the four quadrants approximately 20 mm from the fovea.

Best Disease Eyes

Acquisition of eyes from a 93-year-old donor with a Y227N bestrophin mutation was described in a previous report. Eyes from a second 86-year-old donor with a different mutation (Thr6Arg) were obtained from the Nebraska Lions Eye Bank. The right eye of the latter donor was phthisical and was not used for these studies. On gross examination, the left eye exhibited a subfoveal disciform scar. A wedge from this eye extending from the ora serrata through the fovea and including the scar was fixed for transmission electron microscopy in one-half strength Karnovsky fixative. A second wedge that included portions of the macula but excluded the foveal centralis was collected and fixed in 4% formaldehyde in PBS. Extramacular RPE-choroid was collected for Western blot analysis and for other studies.

This donor was part of the pedigree depicted in Figure 1 and was the father of a Best disease eye donor previously described as having massive accumulations of RPE lipofuscin. All procedures conformed to the Declaration of Helsinki and were performed with the informed consent of the donor families.

Immunohistochemistry

Immunofluorescence studies were performed as described previously. Briefly, sections were blocked by immersion in a solution consisting of 1 mg/mL bovine serum albumin in PBS for 15 minutes. Sections were then incubated in the primary antibody solution for 1 hour, followed by rinsing in PBS and incubation in the appropriate Alexa-488 – conjugated secondary antibodies (Invitrogen, Eugene, OR). Sections were counterstained with either 4’6-diamidino-2-phenylindole (DAPI) or To-Pro-3 (both from Invitrogen), washed at least 3 times for 5 minutes each in PBS, and coverslipped (Aquamount; Ted Pella, Redding, CA). Antibodies used for immunohistochemistry included anti-bestrophin (directed against a C-terminal peptide, ab14929; Novus Biologicals, Littleton, CO) and Abcam used at a concentration of 7.5 µg/mL; anti-glial fibrillary acidic protein (GFAP; Neomarkers, Fremont, CA) used at a concentration of approximately 3 µg/mL; anti–CD29/B1 integrin (clone P5D2; Developmental Studies Hybridoma Bank, Iowa City, IA) at a dilution of 1 µg/mL; anti–serum amyloid P component (1:200 dilution; Neomarkers); anti–vitronectin (5 µg/mL; Ab 19014; Chemicon, Temecula, CA); anti–terminal complement complex (1.5 µg/mL; Dako, Carpenteria, CA). Biotinylated peanut agglutinin (Vector Laboratories, Burlingame, CA) was used to label cone matrix sheaths according to methods described previously. Sections were viewed on either a microscope (BX41; Olympus, Tokyo, Japan) or a confocal microscope (1024 MRC; BioRad). When comparing macular and extramacular regions within a specific donor eye, confocal microscopy was used with identical laser intensity, iris, and gain settings for the two regions compared.

For experiments to assess CD68 immunoreactivity, a prediluted anti–CD68 antibody (Chemicon) and a staining kit (Elite; Vector Laboratories) kit were used as described previously. Sudan black B staining was performed by incubating sections with freshly generated 1% Sudan black B (Fisher Biotech, Pittsburgh, PA) diluted in 70% ethanol for 10 minutes, followed by brief rinsing in 70% ethanol, rinsing in dH2O, and coverslipping (Aquamount; Ted Pella).

Other special pathology stains, including periodic acid Schiff stain, Alcian blue, and Congo red, were performed at the FC Blodi Eye Pathology Laboratory (Department of Ophthalmology and Visual Sciences, University of Iowa, Iowa City, IA).

FIGURE 1. Pedigree of a family with a T6R bestrophin mutation. I–IV is the donor studied in this report. Histopathologic characteristics of II–6 were described previously.
Transmission Electron Microscopy
The macular regions of the Y227N and the T6R eyes were fixed in half-strength Karnovsky fixative (2.5% glutaraldehyde, 2% paraformaldehyde, 2.5% CaCl₂, in 100 mM cacodylate buffer, pH 7.4) for at least 24 hours. Samples were then postfixed in 1% osmium tetroxide/1.5% potassium ferrocyanide in 100 mM cacodylate for 2 hours on ice. Tissues were washed in 100 mM cacodylate buffer, stained en bloc with 2.5% uranyl acetate for 20 minutes, and dehydrated through a graded series of alcohols (50%-100% ethanol). After preparation of the tissue specimens in an equal mixture of 100% ethanol with the transitional solvent propylene oxide (2 × 15-minute steps) and two 15-minute incubations in 100% propylene oxide, tissues were infiltrated in a 2:1 mixture of propylene oxide/Spurr resin, followed by a 1:2 mixture of these components and, after 4 hours in resin alone, were embedded in Spurr resin and polymerized at 60°C for 48 hours. Thin sections were collected on polivynil formal resin (Formvar; SPI Supplies, West Chester, PA)—coated slotted grids using an ultramicrotome (Reichert Jung Ultracut E; Leica Microsystems Nussloch GmbH, Wetzlar, Germany). Grids were then viewed with a transmission electron microscope (JEM-1230; JEOL, Tokyo, Japan) equipped with a digital camera at the University of Iowa Central Microscopy Research Facility.

Quantitative RT-PCR
To determine RNA expression levels of the bestrophin gene (Best1/VMD2), 4-mm trephine punches containing RPE and choroidal cells were collected from the temporal quadrant of three donor eyes. Macular punches were collected at a distance of 2 to 6 mm from the fovea, and peripheral punches were collected at a distance of 10 to 14 mm from the fovea, as described previously.29 Donor ages were 46, 57, and 76 years. Samples were collected and frozen in liquid nitrogen and were then stored at −80°C. Total RNA was extracted (RNeasy kit, Qiagen, Valencia, CA) and was digested with endonuclease (RNase-free DNase; Qiagen). cDNA was synthesized in a random primed reaction using reverse transcriptase (Superscript III; Invitrogen; Carlsbad, CA). Transcript levels were determined by real-time PCR using a sequence analyzer (ABI model 7700; Applied Biosystems, Foster City, CA) and master mix (Q-SYBER Green; BioRad, Hercules, CA). Each sample was analyzed in triplicate, and melting curves were created to confirm that only a single reaction product was formed. VMD2 expression levels were normalized to those of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Primer sequences are depicted in Table 1.

Western Blot Analysis
Western blotting was performed as described previously.29 Briefly, punches from the macular or extramacular RPE-choroid were collected from human donor eyes and were homogenized using Kontes pestles (Chemicon). Antibody Characterization
The bestrophin polyclonal antiserum used in this study was evaluated for its ability to bind the same molecular-weight band as monoclonal antibody E6-6. For these experiments, 10 μg porcine RPE-choroid protein was separated on 10% polyacrylamide gels, as described. Blots were cut in half and were probed with either E6-6 or Abcam polyclonal antibody. To further confirm that the polyclonal antibody recognizes bestrophin, a partial bestrophin clone (clone 3877806) was obtained from Open Biosystems (Huntsville, AL). Escherichia coli containing the pCMV-SPORT 6 plasmid was grown on LB plates with ampicillin, colonies were picked and grown, and plasmids were isolated using the endonuclease (RNase-free DNase; Qiagen). cDNA was added to Cos7 cells (American Type Culture Collection, Manassas, VA), which were harvested after 24 hours. Twenty micrograms Cos7 protein was used in Western blot analysis for detection with anti-bestrophin antibody, as described. Protein extract from transfected and untransfected Cos7 cells were preadsorbed with bestrophin antibody for some immunohistochemistry experiments.

RESULTS
Histopathology of an Eye with a T6R Mutation in the Bestrophin Gene
The peripheral retina of the eye with a T6R bestrophin mutation was within normal limits on histologic examination (Figs. 2A, 2B). Some peripheral drusen were present in Bruch’s membrane, but these were not remarkable for a donor of this age.
Within the vascular arcades but inferotemporal to the foveal avascular zone, we observed accumulations of pigmented cells within the subretinal space overlying a largely intact RPE monolayer (Figs. 2C, 2D). In these areas, focal loss of inner and outer segments and outer nuclear layer attenuation was observed, with a corresponding increase in GFAP labeling in these areas of photoreceptor loss (Fig. 2E). These cells, which are presumably melanophages but may be dystrophic RPE, were less autofluorescent than the underlying RPE and were immunoreactive with an anti–CD68 antibody (Fig. 2F).
More centrally, an area of disciform scarring was present in which the RPE and the outer nuclear layer had undergone degeneration. Bruch’s membrane was intact throughout the specimen, and there was no evidence of choroidal neovascularization. A space was observed between the outer edge of the atrophic neurosensory retina and Bruch’s membrane that was most likely artifactual. One characteristic observed in Best disease that is unusual in normal aging and in AMD was the presence of round or amorphous bodies embedded in the outer retina in the area of scarring (Figs. 2G-J). This material was moderately autofluorescent on ultraviolet light excitation, stained intensely with Sudan black B, and was moderately immunoreactive with antibodies directed against vitronectin, amyloid P component, and terminal complement complex (depicted in Fig. 2I). On standard histologic staining, these deposits were periodic acid Schiff (PAS) positive (Fig. 2H), PAS-reactive after diastase treatment, Alcian blue negative, Congo red negative, and blue on Masson trichrome. In some cases these structures appeared to be budding off Bruch’s membrane, but their appearance and presence in the outer retina was atypical for age-related drusen.

Table 1. Primers Used during Quantitative PCR

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<tr>
<th>Forward (5’–3’)</th>
<th>Reverse (5’–3’)</th>
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<tr>
<td>VMD2</td>
<td>ACCGTTGGAAGACGACCGTTTAT AAGCCAGGCTTGATTGACA</td>
</tr>
<tr>
<td>GAPDH</td>
<td>TGTTGCGCATAGATGACCCCCTT CTGGAACGACATCTGACGG</td>
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Eyes from patients with T6R and Y227N Best disease showed macular scarring on transmission electron microscopy, without evidence of neovascularization. In both cases, Bruch’s membrane was intact over an area of photoreceptor dropout and gliosis (Fig. 3A). Rare inclusions were present in the retina of the T6R donor that resembled lipofuscin on 1-μm sections (Fig. 3A) and were amorphous and electron dense by transmission electron microscopy (Fig. 3B). The RPE was completely atrophic in these areas. Peripheral to the scar, in the donor...
with a T6R mutation, the RPE was remarkable for lipofuscin accumulation. Lipofuscin granules occupied almost the entire cell volume in this case (Fig. 3C), which is similar to results previously reported for a family member. However, this lipofuscin accumulation was not noted for a donor of advanced age with a Y227N mutation (Fig. 3D). The RPE in both cases showed a loss of basal infoldings with moderate basal laminar deposits, and the Y227N eye showed loss of apical microvilli, with hypertrophied rough and smooth endoplasmic reticulum. Some intact junctional complexes were observed in this donor (Fig. 3E). Abnormalities in the choriocapillaris, including loss of fenestrae, presence of vacuoles, and presence of “ghost” vessels, were also noted in both eyes beneath areas of degenerated RPE (data not shown).

**Immunohistochemical Distribution of Bestrophin in the Macular and Peripheral RPE**

Assessing control eyes for bestrophin distribution suggested that immunolabeled bestrophin was more highly expressed in the extramacular than the macular RPE basolateral membrane. This pattern was noted in 18 of 22 eyes (Table 2; Fig. 4). Of the four eyes that did not show increased peripheral labeling, one showed no immunoreactivity in either region, and two of the remaining three had neovascular AMD. In some cases, no labeling of the macular RPE was observed, whereas in other eyes comparatively weak labeling was observed (Figs. 4A-F). Rarely, macular RPE showed intracellular or apical labeling (Fig. 4A). The periphery-to-macula gradient observed for bestrophin was not observed for CD29/β1 integrin, a distinct basolateral RPE protein that showed the increased peripheral labeling observed for bestrophin was not a feature of all basolateral RPE membrane proteins. Preincubation of recombinant bestrophin with anti-bestrophin antibody reduced basolateral RPE labeling on tissue sections (Supplementary Figs. S1A, S1B, online at http://www.iovs.org/cgi/content/full/48/7/3372/DC1).

**Biochemical Assessment of Bestrophin in the Macular and Peripheral RPE**

To confirm the increased peripheral protein expression that we detected immunohistochemically, we performed Western blot analysis using an anti-bestrophin antibody on macular punches and peripheral punches of RPE-choroid from the eyes of nine donors. The major reactive band present in RPE-choroid had an apparent molecular weight of approximately 62 to 65 kDa. This band was not detected in retinal extracts (Fig. 5A). Proteins extracted from the RPE-choroid of the donor eye harboring a T6R bestrophin mutation were also blotted and probed with the bestrophin antibody (Fig. 5B). The dominant band was unchanged compared with that in control donors, though labeling of a smaller band (~47 kDa), possibly a degradation product of bestrophin, was increased. However, this band was observed in controls when longer exposures were obtained. When macular and peripheral regions were compared by Western blot, an elevated level of the major bestrophin band was observed in the peripheral, compared with the macular, RPE samples, in eight of nine cases. In some eyes, this difference was subtle; in others, it was striking. Densitometry was performed on Western blot analysis using the gels tool in ImageJ software. Elevated values in the extramacular RPE-choroid generally ranged from 10% to 390% higher than in macular samples.

For two eyes, RPE-choroid extract from macular, temporal, inferior, nasal, and superior regions was probed separately (Fig. 5C). To confirm that comparable levels of protein were loaded, blots were also probed with anti-RPE65 monoclonal antibody. Unlike bestrophin, RPE65 did not show an elevation in the peripheral samples (Fig. 5D).

The major band in human and porcine RPE-choroid recognized by the bestrophin polyclonal antibody had a molecular weight identical to that of the major band recognized by E6-6 (Supplementary Fig. S1C). Protein extract from Cos7 cells transfected with a plasmid containing a partial bestrophin sequence showed intense reactivity of a group of bands at the appropriate molecular weight for the open reading frames present in this sequence (Supplementary Fig. S1D).

**Distribution of Bestrophin RNA in the Macular and Peripheral RPE**

To verify the relative paucity of bestrophin protein in the macula, we performed quantitative RT-PCR on three samples of RPE-choroid obtained from macular and peripheral regions of the same three donors. Increased levels of bestrophin in the peripheral RPE could also be demonstrated at the transcriptional level. Quantitative PCR of RNA derived from RPE and choroid cells collected from three human donors without macular disease demonstrated that the levels of VMD2 transcripts were more abundant in the peripheral RPE than in the macula. After normalization to the level of GAPDH expression to account for differences in cellularity of the samples, efficiency of the reverse transcription reaction, or other sample variables, the data showed peripheral/macular ratios of 2.4, 2.7, and 2.8 in the three samples (P = 0.03, paired t-test), suggesting that the differences in protein levels detected histochemically and by Western blot were the result of significantly higher levels of VMD2 transcripts in the peripheral RPE than in the central RPE (Fig. 6).

**Discussion**

Best disease is a form of human macular degeneration. The molecular mechanism by which certain retinal diseases primarily affect the macula while others spare the macula is not well understood. There are numerous anatomic differences among the macular and extramacular retina, RPE, and choroid. Relevant biochemical and histochemical correlations have been made as well. The observations that lipid accumulation in Bruch’s membrane, the structural properties of the elastic...
lamina of Bruch’s membrane, and the expression of ICAM-1 in the choriocapillaris differ between macular and peripheral regions might explain the propensity of degenerative events to occur in this region.\(^{29,36,37}\) Environmental challenges faced by the RPE are also different in the macula. In models of light-induced retinal injury, the most severe damage is in the macula, within 5° of the fovea.\(^{38}\) In addition, the relatively high cone photoreceptor density in the macula\(^ {39,40}\) indicates that the phagocytic requirements of the RPE differ in this region.

Best disease is a form of dominant macular dystrophy, and we anticipated that bestrophin expression might be highest at the site of the abnormality (i.e., the macula). Surprisingly, we observed that immunohistochemical labeling of the RPE in human eyes was generally higher in the peripheral than in the macular regions, and this macular-peripheral difference was confirmed by Western blot and quantitative PCR. One interpretation of these findings is that Best disease may represent a 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, where the margin of error is lower. In this model, the peripheral RPE may remain effective at maintaining the ionic milieu of the subretinal space, even with only one functional copy of the VMD2 gene, because of the naturally elevated expression level of bestrophin in this region. In contrast, loss of one functional allele may result in insufficient bestrophin protein in the macula as a consequence of lower rates of synthesis in this region, with corresponding deficits in ion homeostasis. This model may in part explain the macular predilection of Best disease.

This possibility is reinforced by the finding that mutations that result in bestrophin-splicing defects—and that are expected to have a more severe effect than missense mutations associated with Best disease—are associated with a relatively severe blinding condition with more widespread abnormality, autosomal dominant vitreoretinochoroidopathy (ADVIRC).\(^ {41,42}\) Hence, the topographically nonoverlapping patterns of disease caused by ADVIRC-associated mutations and Best disease-associated mutations might be explained by the severity of the mutations and the pattern of bestrophin expression. Recent molecular studies describing the regulatory elements that control bestrophin expression offer further understanding of the topography and regulation of bestrophin expression.\(^ {15}\)

The characteristic vitelliform lesion of Best disease was described in Best’s original report in 1905,\(^ {4}\) but its anatomy

**Figure 4.** Immunohistochemical detection of bestrophin protein (green) in the macular (A, C, E) and peripheral (B, D, F) RPE-choroid of three donor eyes by confocal microscopy. Note that labeling of the basolateral membrane of the RPE is generally much higher in the peripheral than in the macular regions. Comparison of bestrophin (G, H) and CD29/β1 integrin (I, J) labeling of the basolateral RPE in the macular (G, I) and peripheral (H, J) regions of a normal eye from a 74-year-old donor reveals that the relative depression of macular bestrophin is not a general finding for all basolateral RPE markers. Orange-yellow labeling is autofluorescence of RPE lipofuscin, and blue labeling represents To-Pro-3 nuclear counterstain. CC, choriocapillaris. Scale bars, 20 μm.
and composition are still obscure. Much of the yellow material must lie in an extracellular location because the material tends to evolve into a two-phase configuration (yellow material below, serous fluid above) with the passage of time. Recent optical coherence tomography studies suggest that at least one of the extracellular compartments occupied by the yellow material is the subretinal space. It is difficult to understand how patients with Best disease can maintain excellent visual acuity for years if their photoreceptor outer segments are separated from the underlying RPE by this vitelliform material. Whatever the mechanism, we suspect that it will turn out to be the same as that of another mysterious disease, central serous retinopathy, which is characterized by surprisingly good vision despite the relatively high photoreceptor density, fewer phagosomes are observed in the RPE, suggesting that cone outer segment turnover is a relatively slow phenomenon. As a result, it is likely that immediate juxtaposition of the retina with the RPE is less critical in the macula than it is elsewhere in the retina.

Eyes with Best disease may develop choroidal neovascular membranes as an end-stage complication. However, in a recent clinical study, Chung et al. noted that the presence of blood in the subretinal space in Best disease has a more favorable prognosis than it does in neovascular AMD and that the nature of the vascular leaking in Best disease may be distinct from that typically observed in AMD. Although the lack of neovascularization in a small sample of Best disease eyes does not prove that neovascularization in Best disease has a different etiology than in AMD, it is interesting that in the Best disease eyes we examined, Bruch’s membrane was intact and showed no evidence of choroidal neovascularization.

We also made observations on the ultrastructural appearance of the macular RPE in eyes from two distinct genetic forms of Best disease (T6R and Y227N). The patient with the T6R mutation was the father of a 28-year-old eye donor described in a previous report, who was found to have remarkable accumulations of lipofuscin in the RPE. The donor in the current report with a T6R mutation similarly exhibited substantial accumulations of lipofuscin within the RPE (Fig. 3). In contrast, transmission electron microscopy of an eye from a donor with the Y227N mutation, who had clinical manifestations of Best disease with peripheral flecks after the sixth decade of life, showed typical lipofuscin accumulation in the RPE of a 93-year-old donor (Fig. 3). There are at least two explanations for the discrepancy in the appearance of these samples. First, it is possible that different mutations in the VMD2/Best1 gene result in different phenotypes. For example, different mutations in the rds/peripherin gene can alternatively cause peripheral or macular retinal degeneration. Similarly, in the case of VMD2, numerous missense mutations have been described in patients with Best disease, whereas mutations that affect splicing are associated with ADVIRC. It is also possible that the physiological effects of distinct mutations that cause Best disease may be variable. This possibility is borne out by in vitro studies in which the W93C and the R218C mutations have distinct effects on the kinetics of calcium-channel activation and inactivation, though with a similar outcome. The second possible explanation for the dissimilarity in the lipofuscin accumulations among patients with different bestrophin changes is that one or more modifier genes are likely to greatly affect the expressivity of Best disease. For example, some patients with a specific bestrophin mutation within a family have vitelliform lesions from a very young age, whereas in others with the same mutation, vitelliform lesions...
may develop only with advanced age.49,50 Hence, the presence of modifier genes that affect the expressivity of specific bestrophin mutations is inferred. The identification of these modifier genes and the mechanism(s) by which they affect Best disease expressivity will lead to better understanding of the disease process and to improved diagnosis and treatment.

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References


