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Clinicopathologic findings in Best vitelliform macular dystrophy

Qing Zhang,

Department of Ophthalmology, Emory University School of Medicine, Atlanta, GA, USA

Central South University Second Xiangya Hospital, Changsha, Hunan, China

Kent W. Small, and

Molecular Insight LLC, Los Angeles, CA, USA

Hans E. Grossniklaus

Department of Ophthalmology, Emory University School of Medicine, Atlanta, GA, USA

Montgomery Ophthalmic Pathology Laboratory, BT428 Emory Eye Center, 1365-B Clifton Road, Atlanta, GA 30322, USA

Hans E. Grossniklaus: ophtheg@emory.edu

Abstract

Purpose—To correlate the clinical and histopathologic features of Best vitelliform macular dystrophy (BVMD).

Methods—Two eyes were obtained postmortem from a patient with BVMD. The patient's clinical information was reviewed. Series sections of the globes were performed and sequentially stained with hematoxylin-eosin, periodic acid-Schiff or Masson trichrome. A section of the left macula was submitted for electron microscopic processing. Histopathologic findings were reconstructed in a scaled two-dimensional map and compared with fundus photography, fundus autofluorescence (FAF), fundus fluorescein angiography (FFA) and optical coherence tomography (OCT) images.

Results—The macular lesion of the right eye was identified as a well-demarcated region with pigment, elevated submacular yellow material and subretinal fluid. This corresponded histopathologically to a well-circumscribed area of RPE hyperplasia, accumulation of lipofuscin in the RPE, deposition of granular material in the photoreceptors, macrophages and drusen. The left eye displayed a 1 disc diameter chorioretinal scar with surrounding shallow fluid and submacular pigment. This corresponded to RPE changes and a fibrocellular proliferation in the choriocapillaris.

Conclusion—Histopathologic mapping revealed retinal edema, RPE abnormalities, drusen and a chorioretinal scar in BVMD that correlated with the fundus, FFA, FAF and OCT findings.

Keywords

Best vitelliform macular dystrophy; Optical coherence tomography; Fundus autofluorescence; Fundus fluorescein angiography; Two-dimensional reconstruction; Clinicopathologic correlation

Introduction

Best vitelliform macular dystrophy (BVMD; VMD2; Best's disease) is a bilateral, symmetric, progressive disease of the macular area with long-standing loss of vision. Its onset is usually early in life, often by 10 years of age. In 1981, Mohler and Fine [1] described a "0–IV stage" clinical classification of BVMD, while Gass [2] has classified it into six phenotypic stages. Although the phenotypic appearance varies with the stage of the disease, BVMD is characterized by a circumscribed, yellow-orange, vitelli-form (resembling an egg yolk) lesion, which may be single or multiple and 0.5 to 3.0 optic disc diameter in size, and later develops to a deeply and disinterestedly pigmented "scramble-egg" appearance. There is a variable amount of subretinal material that may settle creating a layer, resembling a hypopyon, which is termed pseudohypopyon. As the disease progresses, the macular RPE shows atrophic changes and clumps of pigment, with a gradual visual acuity loss. The evolution of the disease during this period is determined by whether or not new blood vessels invade the abnormal deposit from choroid. Once choroidal neovascularization (CNV) occurs, there may be subretinal hemorrhages and fibrosis (disciform scar), and the visual outcome is poor.

Clinical features of BVMD have been previously reported, such as electro-oculogram (EOG), electroretinogram (ERG), and short-wavelength fundus autofluorescence (FAF) findings. Recent studies have attempted to better identify the functional and anatomic changes of the fundus using optical coherence tomography (OCT). In this study, we report a BVMD case with clinical information, including the fundus appearance, EOG, FAF, FFA and OCT. We correlated the clinical findings with the histopathologic features, including a 2-dimensional topographic reconstruction of the pathologic findings.

Case report

The donor (Fig. 1, arrow) had a history of "congenital" macular lesions with gradually decreased visual acuity in both her eyes, and was subsequently diagnosed as having Best disease by an abnormal EOG. At age 80 years, her visual acuity was 20/400 OD and 20/150 OS, with intermittent horizontal diplopia. No distortion, flashes, or floaters were noted. The patient had monocular double vision in her left eye, which was probably related to the macular disease. The intraocular pressures were 17 mm Hg OD and 14 mm Hg OS. Slit-lamp examination of both eyes was normal, with the exception of 1–2+ nuclear sclerosis. Funduscopic examination showed that vitreous, disc, and retinal vessels in both her eyes were normal. The right eye displayed a "vitelliruptive" stage lesion: the macula had some fine pigment granularity, shallow subretinal fluid and an elevated vitelliform lesion with yellowish material in the inferior portion of the lesion (Fig. 2a). The left eye at "atrophic" stage showed a deep punched-out chorioretinal scar with hyperpigmentation. In the superior portion of the lesion, yellowish material and alteration in the color at the level of the RPE was evident (Fig. 2b). The yellowish material was hyperautofluorescent on FAF examination (Fig. 2c,d). FFA images displayed patchy hyperfluorescence in the macular lesion with areas of blocked-fluorescence in both eyes (Fig. 2e,f). On OCT examination, both eyes contained macular lesions and subretinal fluid (Fig. 2g,h). A single intravitreal injection of 1.0 mg bevacizumab was performed in her left eye. Visual acuity was stable during the follow-up period with no significant functional improvement. The patient had two children and three grandchildren diagnosed with BVMD through EOG and fundus examination. The patient died at age 82 years of heart attack, and the eyes were obtained for post-mortem examination.

Methods

The right eye was fixed in 10% neutral-buffered formalin, and the left eye was fixed in 2.5% glutaraldehyde and transferred to formalin after dissection. The central portion of the eyes in the plane of the pupil, optic nerve, and macula were dehydrated in increasing concentration of alcohol, cleared in xylene and embedded in paraffin. Serial 8 micron thick sections through the posterior pole were made using a microtome (Finesse, Thermo Shandon, Astmore, UK), numbered and sequentially stained with hematoxylin–eosin, periodic acid-Schiff, Masson trichrome or left unstained. The slides were sequentially examined using a microscope (Olympus BH-2, Olympus, Tokyo) with a standard reticule, and a 2-dimensional topographic reconstruction map was performed as previously described [3]. For the evaluation of autofluorescence, unstained sections were performed using an Olympus fluorescent microscope with a 488 nm wavelength laser (Olympus DP10, Tokyo, Japan). The dissected tissue of the macula of the left eye was prepared for transmission electron microscopy (TEM) by fixation in 2.5% glutaraldehyde, postfixation in 2% osmium tetroxide, dehydration, embedding in an epoxy resin (Epon-Araldite), sectioning and then staining with toluidine blue.

Results

Gross examination

The right eye measured 24×23.5×22 mm with 6.5 mm optic nerve attached. The cornea measured 11×10.5 mm. The pupil was round and measured 3.5 mm in diameter. Transillumination was normal. The eye was dissected horizontally. Internal examination showed that the cornea, iris and ciliary body were normal. A 3-piece posterior chamber intraocular lens (PCIOL) with lens remnants was present in the capsule. The vitreous was posteriorly detached. A well-circumscribed, slightly elevated lesion with an egg-yolk appearance was present in the macular area and measured 3 disc diameters. The fovea was identified, and yellowish material was present in the subretinal space (Fig. 3a). The optic nerve head, choroid, and sclera were normal. The left eye measured 23.5×23.5×22 mm with 8 mm optic nerve attached. The cornea measured 11×10.5 mm, and the brown iris contains a 3×3 mm round pupil. Transillumination is normal. The eye was dissected horizontally. Internal examination showed that the cornea, iris and ciliary body were normal. A 3-piece PCIOL was present in the lens capsule. The vitreous was posteriorly detached. The macula was atrophic and scarred with pigmentation present in the parafoveal area. Yellowish material and alteration in the color of the RPE was observed in the superior portion of the lesion. The optic nerve head, choroid, and sclera were normal (Fig. 3b). A 2×2 mm section of the macular area was dissected and submitted for transmission electron microscopy (TEM) processing.

Light microscopic examination

The anterior segment of the right eye was normal except for a portion of a PCIOL with cataractous lens remnants. Sections through the macular area displayed that the ganglion cell layer and the inner and the outer plexiform layers were edematous (Fig. 4a). The peripheral retina showed typical peripheral cystoid degeneration (TPCD). In the macular area, just beneath the zone of the photoreceptor degeneration, the RPE cells were slightly larger than normal, and the cell density was higher than the adjacent area (Fig. 4a). The cytoplasm was filled with PAS-positive granular lipofuscin that was most prominent in the macula, but also in the periphery. Hyperplastic RPE cells were also seen adjacent to the optic nerve. Macrophages with PAS-positive deposits and pigmented granules were present in the subretinal space and also in the outer photoreceptor segments (Fig. 4b). Within the macula, there were multiple drusen with PAS-positive material (Fig. 4c,d,e). This material

corresponded to the central yellow deposits noted clinically. Bruch's membrane was intact, except in the region of the large sub-RPE drusen (Fig. 4d,e). In addition, there was an epiretinal membrane measuring approximately 1,600 microns in diameter temporal to the optic nerve (Fig. 4f). The sclera and optic nerve was normal. In the left eye, the cornea, iris, ciliary body was normal. A 3-piece IOL was present in the posterior chamber. There was a 2×2 mm posterior defect due to dissection for TEM processing. The remaining retina was mildly atrophic, and the peripheral retina showed TPCD. There were areas with hyperplastic RPE with lipofuscin and also areas with atrophic RPE posteriorly. No choroidal neovascularization was found. These findings were topographically located in the two-dimensional reconstruction (Fig. 5) as previously described [3].

Fluorescent microscopic examination

Fluorescent microscopic examination of the unstained sections of the right eye disclosed brilliant-green autofluorescence within the RPE, drusen, and the macrophages in the outer retina and subretinal space (Fig. 6).

Electron microscopic examination

Ultrastructural evaluation showed Bruch's membrane was intact in the area examined (Fig. 7a). There were fewer melanosomes but more lipofuscin granules than normally observed in the RPE (Fig. 7b). These cytoplasmic granules were evident throughout the RPE and varied in size and shape, containing relatively homogeneous material of intermediate electron density. The melanosomes in RPE cells were opaque and either round or oval in cross-section (Fig. 7c). Wide-space collagen was present. Some of the macrophages identified in the subretinal space also contained intermediate electron dense granules (Fig. 7d). There were focal areas of chorioretinal fibrocellular proliferation. Small, and irregular, electron-dense particles were observed in the extracellular space of the choroid.

Discussion

There is limited pathologic information concerning BVMD. In 1950, Klien [4] examined the eyes of a BVMD donor, and demonstrated that the early subjective symptoms of the disease corresponded with the extensively degenerative retinal changes. Frangieh [5] also noted that the sensory retina may be the primary site of the disease process because the RPE was mostly intact, whereas the photoreceptor cell layer was found to be severely affected in the case they studied. Weingeist [6] examined a younger BVMD patient who had advanced disease. The author, however, suggested that this entity should be set apart from the other primary retinal degenerations by the preservation of visual acuity, color vision, and dark adaptation with a decreased EOG light peak–dark trough ratio, which indicates RPE abnormalities in the early stage. O'Gorman [7] studied the light and electron microscopic features of BVMD, and concluded that BVMD appears to be a disorder of the degenerated RPE that secondarily affects focal areas of the retina.

In our current study, we documented both clinical and pathologic findings from a patient with BVMD with both eyes in different stages of the disease. We found the following features: RPE hyperplasia in the macula, accumulation of abnormal lipofuscin in RPE cells, deposition of granular material in the photoreceptor area, and chorioretinal scarring that corresponded with the clinical findings. Our studies showed that there was an accumulation of autofluorescent material in the outer retina and the subretinal space. This material has been thought to represent indigestible components of outer segments that accumulate because of the lack of direct apposition of the outer segments to the RPE. Eventual phagocytosis of these older outer segments over time would load the RPE cells with material known to have precursors of lipofuscin, and may account for why lipofuscin is found in

large amounts of RPE in BVMD. An important constituent of the lipofuscin is A2E. Precursors of A2E, including A2-PE-H2, A2-PE, and A2-rhodopsin, which are also autofluorescent, form in outer segments prior to phagocytosis by the RPE, and suggest that autofluorescence does not necessarily arise only from the RPE [8]. OCT technique provides cross-sectional images of the various layers of the neurosensory retina and the RPE by virtue of in vivo microscopy. Most of OCT studies have shown that both early and late lesions of BVMD have subretinal fluid [9], as was present in our case. This clear and colorless subretinal fluid was considered as the underlying defect in BVMD by elevating the retina away from the RPE and making phagocytosis of the outer segments less likely to occur. The mutation in the gene coding for bestrophin-1, a Ca^{2+} -sensitive Cl^{-} channel protein located in the basolateral plasma membrane of the RPE [10], may directly or indirectly decrease RPE cell function, pumping the fluid from the subretinal space.

Several key features have been described in BVMD. Previous studies have found RPE and retina abnormalities in the central macula, but these cases had advanced disease and sometimes concurrent CNV, limiting the ability to study the primary effects caused by BVMD. Future cases should document this entity from early to late stages utilizing different techniques, including EOG, FFA, FAF, and OCT to determine the pathophysiologic and biochemical conditions of BVMD. Given the lack of suitable autopsy sample, the establishment of RPE cell lines from such affected eyes may be helpful for providing experimental material.

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Fig 1.
Pedigree of family with Best vitelliform macular dystrophy. *Arrow* indicates proband;
square, male; *circle*, female; *solid square or circle*, presence of BVMD

Fig 2.

Clinical examination. **a** Fundusphotograph shows the “vitelliruptive” stage in the right eye. The macula has some fine pigment granularity, shallow subretinal fluid and an elevated vitelliform lesion with yellowish material (*arrows*) in the inferior portion of the lesion. **b** The left eye at “atrophic” stage of BVMD contains a deep punched-out chorioretinal scar with hyperpigmentation (*arrows*). In the superior portion of the lesion, yellowish material and alteration in the color at the level of the RPE (*arrowheads*) is evident. **c,d** FAF images show that the yellowish material is hyperautofluorescent (*arrows*). **e,f** FFA shows no leakage into the yellow deposits. Patchy hyperfluorescence (*arrows*) in the macular lesion with areas of

blocked-fluorescence (*arrowheads*) is present in both eyes. **g,h** On OCT examination, both eyes contain macular lesions and subretinal fluid (*arrows*)

Fig 3. Gross examination. **a** The right eye contains a 3 disc diameter, well-circumscribed, slightly elevated lesion with an egg-yolk appearance in the macula. Yellowish material (*arrows*) is observed in the subretinal space. **b** The left eye shows that the macula is atrophic and scarred with hyperpigmentation

Fig 4.

Light microscopic examination. **a** The ganglion cell layer, the inner and the outer plexiform layers are edematous. The RPE (*arrows*) is slightly larger than normal and contains excessive amounts of PAS-positive granular lipofuscin (PAS, $\times 200$, *inset*, original magnification $\times 400$). **b** Macrophages with PAS-positive deposits are present in the subretinal space with focal dropout of the overlying outer photoreceptor segments (PAS, $\times 100$). **c** There is drusen (*arrows*) with PAS positive material identified within the fovea (PAS, $\times 40$, *inset*, original magnification $\times 400$). **d** Bruch's membrane is intact, except in the region of the large sub-RPE drusen (*arrowheads*; PAS, $\times 100$). **e** The PAS-positive material in drusen (shown in Fig. 4d) stains blue with Masson trichrome (Masson trichrome stain, $\times 100$). **f** An epiretinal membrane (*arrows*) is observed temporal to the optic nerve head (PAS, $\times 100$)

Fig 5. Histologic 2-dimensional reconstruction demonstrates the topographic location of the drusen, macrophages, and epiretinal membrane in the right eye

Fig 6.

Fluorescent microscopic examination of unstained sections of the right eye displays brilliant-green autofluorescence within the macrophages in the outer retina and subretinal space ($\times 100$). **b** Composite image orientates the retina and macrophages ($\times 100$). **c,d** Brilliant-green autofluorescence (*arrows*) is present in the RPE, drusen, macrophages and the outer retina (**c** $\times 100$, **d** $\times 200$)

Fig 7.

Electron microscopic findings. **a** The ultrastructure of RPE, Bruch's membrane and choriocapillary (*asterisk*) are identified. Bruch's membrane (*arrows*) is intact in the examined area ($\times 1,900$). **b** Large accumulations of lipofuscin granules (*arrows*) are present in the RPE. These cytoplasmic granules are evident throughout the RPE and vary in size and shape, containing a relatively homogeneous material of intermediate electron density ($\times 3,600$). **c** The melanosomes in RPE cells are electron-opaque and either round or oval in cross-section. Wide-spaced collagen (*arrows*) is identified ($\times 5,800$). **d** Some of the macrophages in the subretinal space contain intermediately dense granules ($\times 2,900$)