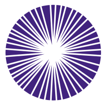

AMERICAN ACADEMY™
OF OPHTHALMOLOGY

Ocular Histopathology and Immunohistochemical Analysis in the Oldest Known Individual with Autosomal Dominant Vitreoretinochoroidopathy

Morton F. Goldberg, MD,[1] Scott McLeod,[1] Mark Tso, MD,[1] Kirk Packo, MD,[2] Malia Edwards, PhD,[1] Imran A. Bhutto, MD, PhD,[1] Rajkumar Baldeosingh, MS,[1] Charles Eberhart, MD,[1] Bernhard H.F. Weber, PhD,[3] Gerard A. Lutty, PhD[1]

Purpose: To assess the immunohistochemical and histopathologic changes in a subject with autosomal dominant vitreoretinochoroidopathy (ADVIRC).

Design: Research manuscript.

Participant: A 92-year-old white man with ADVIRC.

Methods: The subject was documented clinically for 54 years. The retina/choroid complex of the right eye was evaluated with cryosections stained with hematoxylin–eosin or periodic acid–Schiff (PAS) reagent. Cryosections also were evaluated with immunofluorescence or alkaline phosphatase (APase) immunohistochemistry (IHC) using primary antibodies against bestrophin 1 (*BEST1*), glial fibrillary acidic protein (GFAP), pigment epithelium–derived factor (PEDF), RPE65, transforming growth factor (TGF)-β, vascular endothelial growth factor (VEGF), and vimentin. The left retina and choroid were evaluated as flatmounts using immunofluorescence. Ulex europaeus agglutinin (UEA) lectin was used to stain viable vasculature.

Main Outcome Measures: The immunohistochemical and histopathologic changes in retina and choroid from a subject with ADVIRC.

Results: The subject had a heterozygous c.248G>A variant in exon 4 of the *BEST1* gene. There was widespread chorioretinal degeneration and atrophy except for an island of spared retinal pigment epithelium (RPE) monolayer in the perimacula/macula in both eyes. In this region, some photoreceptors were present, choriocapillaris (CC) was spared, and RPE cells were in their normal disposition. There was a Müller cell periretinal membrane throughout much of the fundus. Bestrophin-1 was not detected or only minimally present by IHC in the ADVIRC RPE, even in the spared RPE area. Beyond the island of retained RPE monolayer on Bruch's membrane (BrMb), there was migration of RPE into the neuroretina, often ensheathing blood vessels and producing excessive matrix within their perivascular aggregations.

Conclusions: The primary defect in ADVIRC is in the RPE, the only cells in the eye that express the *BEST1* gene. The dysfunctional RPE cells may go through epithelial/mesenchymal transition as they migrate from BrMb to form papillary aggregations in the neuroretina, often ensheathing blood vessels. This may be the reason for retinal blood vessel nonperfusion. Migration of RPE from BrMb also was associated with attenuation of the CC. *Ophthalmology Retina 2017;■:1–19 © 2017 by the American Academy of Ophthalmology*

The initial sibship with family members affected by autosomal dominant vitreoretinochoroidopathy (ADVIRC) (Online Mendelian Inheritance in Man 193220) was reported 35 years ago.[1] We provided the ADVIRC name at that time[1] and subsequently identified the causative mutation in the bestrophin-1 (*BEST1*) gene.[2] The histopathologic changes included atrophic retinal pigment epithelium (RPE), disorganization of the sensory retina, multifocal loss of photoreceptors, RPE ensheathment of retinal blood vessels, and preretinal membranes.[3]

Since the original publications, approximately 12 unrelated families have been reported with this rare disorder, all of which were associated with different mutations in the *BEST1* gene.[1,4–9] Initially, the progression of ADVIRC seemed limited, but several subsequent reports, including our own, have shown progression of ophthalmoscopic and other ocular disorders to various extents.[2,10]

This report describes the immunohistochemical and histopathologic abnormalities in the fundus tissues of the oldest known individual with ADVIRC (92 years at death). His advanced age and 54-year medical history allow a more complete description of the full spectrum and severity of this disease. This study evaluated the histopathologic progression of ADVIRC and the abnormal expression of BEST1 in the RPE using immunohistochemistry (IHC). The results confirm RPE as the primary site of gene malfunction.

© 2017 by the American Academy of Ophthalmology
Published by Elsevier Inc.

http://dx.doi.org/10.1016/j.oret.2017.08.001
ISSN 2468-6530/17

Methods

All procedures in this study adhered to the tenets of the Declaration of Helsinki regarding research involving human tissue and were approved by the institutional review boards of the Johns Hopkins Medical Institutions. The death to enucleation time for the eyes was 7.4 hours. The right eye was fixed immediately while the left eye was processed 47 hours postmortem. The right eye of this 92-year-old subject was shipped to the Wilmer Ophthalmological Institute in 10% neutral buffered formalin fixation at 4°C, and the left eye arrived in a vial on wet ice. In addition, whole blood from the subject was shipped at 4°C for genomic analysis.

Tissue Preparation

A cap was removed from the nasal side of the right globe, and then the anterior portion of the eye was removed. The cap and anterior eye were embedded in paraffin. The remainder of that eyecup was washed in several changes of 0.1 mol/l cacodylate buffer, pH 7.4, at 4°C. After examination and imaging, the posterior eyecup of the right eye was cut into calottes of the vitreous−retina−choroid complex and cryopreserved with increasing concentrations of sucrose, as previously described.[11] Serial 8-μm−thick cryosections were cut from the cryo blocks, collected in duplicate on glass slides coated with Vectabond (Vector, Burlingame, CA), dried, and stored at −80°C until IHC was performed. Both hematoxylin and eosin, as well as hematoxylin and periodic acid−Schiff (PAS) reagent, were used to stain paraffin sections and cryosections.

The anterior segment of the left eye also was removed after a circumferential incision, approximately 5 mm from the limbus. A dissecting microscope (Stemi 2000; Carl Zeiss, Inc, Thornwood, NY) with a mounted digital camera (Q-imaging, Vancouver, BC) was used for gross examination and photography of the posterior eyecup, and to capture high-resolution images. Images were imported directly into Adobe Creative Suites (ver. 6.0; Adobe Systems Inc, San Jose, CA) on a PowerMac G5 (Apple Computer, Cupertino, CA).

Reflected and transmitted illuminations were used for photographic documentation before dissection. Vitreous was removed, and the sensory retina was then excised from the RPE/choroid in the left eye. After removing the retina, the eyecup containing the choroid, with the RPE intact, was reimaged. The choroid was then dissected from the sclera, washed briefly in 0.1 mol/l cacodylate, and fixed overnight in 2% paraformaldehyde in 0.1 mol/l cacodylate buffer at 4°C.

Immunohistochemistry and Imaging of Retinal and Choroidal Whole Mounts

The choroid of the left eye was fixed, washed in Tris-buffered saline (TBS) with 0.1% TritonX-100, and incubated, as described previously, with fluorescein isothiocyanate-conjugated to Ulex europaeus agglutinin (UEA) lectin (UEA stains blood vessels) for 48 hours at 4°C (Sigma L9006;1:100; St. Louis, MO).[12] Immunofluorescence in the flatmounted choroid was imaged with a confocal microscope (710; Carl Zeiss MicroImaging; Carl Zeiss, Inc) at 488-nm excitation. Overlapping fields (10% overlap) were captured from the submacular region to the temporal peripheral choroid at 2048 × 2048 pixel resolution using Zen Software (2010, Carl Zeiss, Inc). Percent vascular area determinations of choroidal vascular density were made as previously reported.[12]

The isolated retina of the left eye was processed as previously described.[13] Briefly, after overnight fixation, the tissue was washed and blocked overnight in 5% goat serum prepared in TBS with 0.1% Triton X-100 and 0.1% bovine serum albumin. Tissues were incubated in primary antibodies prepared in TBS with 0.1% Triton X-100/bovine serum albumin for 72 hours at 4°C and in secondary antibodies for 48 hours as published previously.[13,14] Peanut agglutinin (PNA) and UEA lectin were applied along with the secondary antibodies on some retinal and choroidal pieces. Antibodies used are listed in Table 1. Images were collected on a Zeiss 710 confocal microscope. After imaging, the retina and choroid were embedded in JB-4 or cryopreserved and sectioned (8-μm sections) for further analysis.

Immunohistochemistry on Sections

Eight-micron cryosections of the ADVIRC right eye were immunolabeled with antibodies using both immunofluorescence and alkaline phosphatase (APase) staining methods as described previously.[15,16] Cryosections from an aged control eye (71-year-old white man) from our archives were run in parallel with the ADVIRC tissue. Briefly, sections for immunofluorescence were air dried and permeabilized in methanol for 5 minutes, then blocked in 2% goat serum for 20 minutes, and incubated in primary antibodies for 2 hours at room temperature as published previously.[16] Secondary antibodies (1:500) and 4′,6′-diamidino-2-phenylindole (1:1000) were applied after washes, and sections were incubated for 30 minutes. Retinal pigment epithelium lipofuscin autofluorescence was quenched using 1% Sudan black B in 70% ethyl alcohol treatment after immunolabeling.[17] After washing, sections were coverslipped with Dako Cytomation (Carpinteria, CA) mounting media. Images were collected on a Zeiss 710 confocal microscope. Melanin in RPE and melanocytes was bleached in sections stained with APase IHC as reported previously.[15]

Genetics

To confirm the diagnosis of ADVIRC, DNA was extracted from blood lymphocytes of this individual for sequence analysis of exon 4 of the *BEST1* gene. This exon had been found in an earlier study to harbor a nonsynonymous variant, c.248G>A (p.Gly83Asp), in his affected son, who was the proband of our initial report.[1−3] The c.248G>A variant was shown to segregate with ADVIRC in the family and to represent a pathologic mutation affecting a highly conserved transmembrane domain in the BEST1 protein.

Results

Clinical Background

The ADVIRC subject was initially examined clinically at age 38 years, and some of his clinical data at age 55 years were published as Case III-3 in our initial article that described the first pedigree of this rare disease and that also provided its name.[1] At age 55 years, the patient's visual acuity was 20/30 in the right eye and 20/20 in the left eye. The peripheral fundi showed an annular distribution of widespread, intense pigmentary abnormalities between the ora serrata and the posterior pole of each eye. Although abnormalities were more prominent in the periphery of the fundi, there was no discrete pigmentary border located along the posterior margin of the degenerated fundus in this subject, unlike several other patients with ADVIRC in his sibship and in others. The right fundus also showed mild to moderate retinal arteriolar narrowing, a gliotic membrane at the disc, and peripapillary areas of RPE atrophy and migration (Fig 1A). Visibility of large choroidal vessels in the peripapillary fundus suggested atrophy of both the choriocapillaris (CC) and the RPE. The peripheral

Download English Version:

https://daneshyari.com/en/article/8794615

Download Persian Version:

https://daneshyari.com/article/8794615

Daneshyari.com