Strong Labeling for Iron and the Iron-Handling Proteins Ferritin and Ferroportin in the Photoreceptor Layer in Age-Related Macular Degeneration

Age-related macular degeneration (AMD) is the leading cause of legal blindness among people 65 years and older. The cause of AMD is unclear, but oxidative stress may play a role because photoreceptors, which are high in readily oxidized polyunsaturated fatty acids, are exposed to high oxygen tensions and photodestruction. Antioxidant vitamins decrease the risk of vision loss in some patients with AMD, supporting the role of oxidative stress in AMD. Iron can cause oxidative stress, and we have found that maculae from patients with AMD have higher iron levels in the retinal pigment epithelium (RPE) and Bruch’s membrane than do maculae from age-matched control subjects. Although elevated iron levels may be associated with AMD but not necessarily causal, recent evidence supports causality; we have found that retinas from iron-overloaded (ceruloplasmin/hephaestin–deficient) mice have some features of AMD and that a patient with retinal iron overload resulting from the rare hereditary disease aceruloplasminemia had early-onset drusen.

Furthermore, elevated photoreceptor iron levels are associated with retinal degeneration in the Royal College of Surgeons rat. We report a case of GA exhibited strong label for iron in the neurosensory retina. This retina also labeled strongly for ferroportin and ferritin, both of which have been found to be up-regulated in response to elevated iron levels in the mouse retina.

Comment. We report a case of GA in a 72-year-old white male postmortem eye donor with advanced GA (Figure 1A) was labeled for iron using the 3,3'-diaminobenzidine–enhanced Perls stain as previously described. Strong labeling was evident in the photoreceptor and internal limiting membrane regions (Figure 1C and D). This is significant because none of the 9 postmortem retinas from elderly donors with normal eyes had detectable iron in the neurosensory retina when stained using the same technique.

Because increased intracellular iron causes an increase in ferritin and ferroportin in nonocular tissues, we sought to determine whether the elevated photoreceptor iron level may be associated with an increase in these iron-handling proteins in the macula with GA compared with a normal macula from a 65-year-old white donor. We found that anti-ferroportin strongly labeled cells in the photoreceptor layer and along the internal limiting membrane (probably Müller cell end feet) in the macula with GA (Figure 2A). In comparison, photoreceptor label in the normal macula had a more limited distribution in the outer plexiform layer (Figure 2B). Similarly, label for the ferritin was present in the photoreceptor and internal limiting membrane regions in the macula with GA (Figure 2C), but only weakly labeled the normal macula (Figure 2D).

Figure 1. Iron in a postmortem macula with geographic atrophy. A, Photograph of the transilluminated eye cup, with the area of atrophy demarcated by arrows. The optic nerve is the white circle on the left. B, Fluorescence photomicrograph of the macula labeled by immunohistochemistry as previously described. Photoreceptors are labeled with anti-rhodopsin (red), nuclei are labeled with 4',6-diamidino-2-phenylindole (blue), and retinal pigment epithelial (RPE) cells are autofluorescent (yellow). The photoreceptors label with anti-rhodopsin in the outer nuclear layer (ONL) and the nerve fiber layer of Henle (H). As expected, cells in the inner nuclear layer (INL) are negative for rhodopsin. C, The photoreceptor layer, particularly in the vicinity of the nerve fiber layer of Henle (H), is positive for the 3,3'-diaminobenzidine–enhanced Perls stain for iron. The section was bleached with hydrogen peroxide as previously described and counterstained with Richardson (methylene blue/azure II) stain. Br indicates Bruch’s membrane; GCL, ganglion cell layer. D, 3,3'-Diaminobenzidine-enhanced Perls stain labeling both the photoreceptor and the internal limiting membrane regions.
role of iron export in AMD needs further investigation, but this report provides evidence that iron overload can occur in AMD not only in the RPE and Bruch’s membrane but also in the neurosensory retina. Because iron can cause oxidative stress, it may be toxic to both the RPE and photoreceptors, cells in which cell death leads to blindness in AMD. Iron chelation therapy may one day prove useful in the prevention of vision loss due to AMD.

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Figure 2. A macula with geographic atrophy (GA) (A and C) and a normal macula (B and D) immunolabeled with anti-ferritin and anti-ferroportin. A, The macula with GA labeled with anti-ferroportin (red). The nuclei are labeled with DAPI (blue). The retinal pigment epithelial cells are autofluorescent (yellow). GCL indicates the ganglion cell layer; ONL, outer nuclear layer. B, The normal macula labeled with anti-ferroportin. C, The macula with GA labeled with anti-ferritin. D, The normal macula labeled with anti-ferritin.

Optical Coherence Tomographic Findings of Combined Hamartoma of the Retina and Retinal Pigment Epithelium in 11 Patients

Combined hamartoma of the retina and retinal pigment epithelium (RPE) is an uncommon fundus tumor with classic clinical features. In 1984, Schachat et al2 published 60 cases collected from the members of the Macula Society and described the clinical features and natural course of this benign lesion. They noted that the lesion was typically pigmented (52 cases [87%]), elevated (48 cases [80%]), and had vascular tortuosity (56 cases [93%]), vitreoretinal interface changes (47 cases [78%]), and lipid exudate (4 cases [7%]). The vitreoretinal interface changes in that series of patients were observed on ophthalmoscopic examination as surface wrinkling retinopathy and also, to some extent, were inferred based on fluorescein angiography as vascular dragging. In their series, progressive loss of visual acuity due to tractional distortion of the fovea from vitreoretinal interface problems was observed in 14 (24%) of 60 cases. The importance of the vitreoretinal interface in this condition and particularly as a risk for visual loss was recognized, but it was not yet completely understood. Speculation existed regarding the depth of the tractional component, whether it involved the surface or deep layers, and controversy arose regarding the benefits of surgical removal of the tractional component.5,6 At that time, high-resolution, cross-sectional analysis of the retina was unavailable for visualization of vitreoretinal interface abnormalities.

Twenty years later, we further investigate the vitreoretinal interface abnormalities and retinal microarchitecture of combined hamartoma of the retina and RPE using optical coherence tomography (OCT). Previously presumed findings or those that were difficult to document, such as vitreoretinal interface abnormalities, can be imaged remarkably well with OCT. In this article, we examined 11 consecutive patients who had combined hamartoma of the retina and RPE using OCT.

Methods. The clinical records of all patients having the diagnosis of combined hamartoma of the retina and RPE and imaged with OCT (Zeiss Stratus OCT model 3000; Carl Zeiss Ophthalmic Systems, Dublin, Calif) on the Ocular Oncology Service at Wills Eye Hospital, Philadelphia, Pa, between January 1, 2003, and April 1, 2004, were reviewed. Institutional review board approval was obtained. Data were gathered regarding clinical, OCT, and ultrasonographic features of the combined hamartoma and the