We performed a histopathologic and immunohistochemical study of eyes obtained at autopsy of an 84-year-old man from a family with X-linked cone degeneration in which affected members have a 6.5-kilobase deletion in the red cone pigment gene. At his most recent ocular examination, at age 71 years, this patient had had a visual acuity of 20/200 OU, fundus changes suggestive of macular degeneration, borderline-normal full-field rod electroretinograms, and profoundly reduced full-field cone electroretinograms. Histopathologic examination demonstrated marked loss of cone and rod photoreceptors and the retinal pigment epithelium in the central macula. The peripheral cone population was reduced, while the peripheral rod population was relatively preserved. Immunohistochemical examination with an antibody to both red and green cone opsin and an antibody to blue cone opsin disclosed a prominent loss of the red and green cone population and preservation of the blue cone population. These findings show that a defect in the red cone pigment gene can result in extensive degeneration of the red and green cone population across the retina.

Hereditary conditions that result in generalized cone dysfunction may be divided into 2 groups. The first group is considered non-progressive and consists of cases recognized near birth because of decreased visual acuity, sensitivity to light, nystagmus, and abnormal color vision. Patients in this group include those with autosomal recessive complete achromatopsia and X-linked blue cone monochromacy. While the retina usually appears normal or nearly normal in early life, the electroretinogram (ERG) demonstrates markedly diminished cone responses with normal rod responses. The second group is considered progressive and consists of cases typically identified in young adulthood because of gradual deterioration of visual acuity and decline in color vision. Patients in this group include those with autosomal dominant, autosomal recessive, and X-linked cone degenerations. The macula may have an abnormal appearance ranging from mild granular changes to geographic atrophy. As in the nonprogressive forms, the diagnosis is confirmed by ERG testing, which shows diminished full-field cone amplitudes with normal or near-normal rod function. No treatments are known for patients with hereditary cone dysfunctions.

We studied eyes obtained at autopsy of an 84-year-old affected man from a family with X-linked cone degeneration. This patient and other affected family members had a known 6.5-kilobase deletion in the red cone pigment gene. To our knowledge, this report is the first histopathologic and immunohistochemical study of X-linked cone degeneration with a known gene defect.

The donor with X-linked cone degeneration was an 84-year-old man whose clinical findings have been previously described (see Reichel et al, patient II-4). At our most recent ocular examination, at age 71 years, he had a best-corrected Snellen visual acuity of 20/200 OU with a 10° central scotoma with full peripheral visual fields on Goldmann perimetry; a nonspecific axis of confusion on the Farnsworth D15 panel; and normal full-field rod ERGs and non-detectable (ie, <10 µV) full-field cone ERGs. His eyes were enucleated 7 ½ hours after he died of cardiac arrest. The donor’s brother also had a visual acuity of 20/200, impaired color vision, normal rod ERGs, and diminished cone ERGs. The donor’s 2 daughters had normal visual acuity and re-
duced cone ERGs. The daughters’ cone responses, although diminished, were still greater than those of their father or paternal uncle. One daughter’s son, at age 15 years, had nearly normal visual acuity (20/30 OU), a protan deficiency on the Farnsworth D15 panel, a normal rod ERG, and a reduced cone ERG.

The right eye was immediately fixed by immersion in 2.5% glutaraldehyde and 1% formaldehyde in 0.1-mol/L phosphate buffer at pH 7.4. The left eye was fixed in 4% formaldehyde in 0.1-mol/L phosphate buffer at pH 7.4 and stored wet at 4°C for immunohistochemical studies.

The right eye was processed for light and electron microscopy and embedded in epoxy resin (Taab 812, Mari- vac, Ltd, Halifax, Nova Scotia) blocks for sectioning. Sections for light microscopy were cut from the macula and peripheral retina at a thickness of 1 µm and stained with a 1:1 solution of 2% methylene blue and 2% azure II. Silver or gold sections from the same regions were stained with uranyl acetate–lead citrate and examined with an electron microscope.

With respect to the left eye, 4-mm trephine punches were taken between 10° and 20° eccentric to the fovea in the near temporal peripheral retina along the horizontal meridian. Retinal punches were then frozen in a pentane slush in liquid nitrogen and embedded in OCT compound (Miles, Inc, Elkhart, Ind) for cryosectioning. Cryostat sections (6 µm) were cut and mounted on slides immersed for 10 minutes in a phosphate-buffered saline (PBS) solution containing 0.3% Triton X-100, blocked with normal goat serum for 20 minutes, and then incubated for 1 hour at room temperature with polyclonal antibodies diluted 1:10 in PBS and specific to either red and green cone opsin in combination or to blue cone opsin. The sections were then rinsed in PBS and labeled with a secondary goat anti–rabbit fluorescent antibody named CY3 (Jackson Immuno- research Laboratories, West Grove, Pa), diluted 1:200 in PBS. Labeled sections were then viewed on an epifluorescent photomicroscope (Carl Zeiss, Oberkochen, West Germany).

For morphological comparison, we also evaluated an eye obtained at autopsy from a 67-year-old man with X-linked cone degeneration, showing an atrophic macular lesion and the optic nerve head with a temporal conus.

**Figure 1. Fundus of the right eye of an 84-year-old man with X-linked cone degeneration, showing an atrophic macular lesion and the optic nerve head with a temporal conus.**

**Figure 2. Light micrograph of the eye obtained at autopsy from the donor affected with X-linked cone degeneration (top), showing an area within the macular lesion where Henle fiber layer abuts Bruch membrane, contrasted with an age-matched control (bottom). Nerve fiber layer is thinned and vacuolated in the patient compared with the control. B indicates Bruch membrane; RPE, retinal pigment epithelium; OS, outer segment; IS, inner segment; ONL, outer nuclear layer; H, Henle fiber layer; INL, inner nuclear layer; IPL, inner plexiform layer; OPL, outer plexiform layer; G, ganglion cell layer; and N, nerve fiber layer. Bar indicates 60 µm.**

**PATHOLOGIC FINDINGS**

Gross examination of the right eye from the patient with X-linked cone degeneration disclosed a large atrophic macular lesion and an unremarkable fundus periphery (Figure 1). Histologically, there was loss of cone and rod photoreceptors, retinal pigment epithelium, and choriocapillaris in the central macula (Figure 2, top) compared with an age-matched control (Figure 2, bottom). In this area the retinal pigment epithelium and outer nuclear layer were absent, and the Henle fiber layer was atrophic and abutted the Bruch membrane. The inner nuclear layer and ganglion cell layer showed a reduction in cell number compared with an age-matched control. Just anterior to these anomalies, abnormal rod and cone pho-
Receptors could be seen by light microscopy (Figure 3, A) and electron microscopy (Figure 3, B). The mid-peripheral retina (Figure 4, A) also showed a reduction in the number of cones compared with the control (Figure 4, B). The retinal pigment epithelium was attenuated over drusen (Figure 4, A). In the peripheral retina there was an absence of cones and relatively preserved rods; the remaining rods showed vacuolization of the inner segments and shortened outer segments. In the peripheral retina, the retinal pigment epithelium showed a scarcity of melanin granules and numerous melanolysosomes; the cells lacked both apical processes and basal infoldings. Drusen were prominent, and the elastica of Bruch membrane was granular and thickened (Figure 5).

Immunohistochemical examination with an antibody to both red and green cone opsins and an antibody to blue cone opsins showed, outside the atrophic macular lesion, a specific loss of many red and green cones with sparing of blue cones. In the patient eye, the antibody to the red and green cone opsins weakly stained remaining inner segments (Figure 6, A) in contrast to the control eye, where this antibody strongly stained outer segments (Figure 6, C). In contrast, antibody specific for blue cone opsins similarly stained the outer segments of the patient and control eyes (Figure 6, B and D).

COMMENT

Our patient with X-linked cone degeneration had fundus changes of macular degeneration, borderline-normal full-field rod ERGs, and profoundly reduced full-field cone ERGs at 71 years of age. If the macular changes were age related and confined to the macula, a normal or borderline-normal full-field cone ERG would be expected, since the majority of the cones are outside the macula. Because of the abnormal full-field cone ERG responses and the history of declining visual acuity and color vision, a progressive generalized degenerative process involving the cones was diagnosed clinically. Our histological findings confirm that this patient with a large deletion in the red cone pigment gene had extensive cone photoreceptor degeneration not only in the macula but also across the retina.

On the basis of the prolonged visual evoked potential latencies in some male patients with X-linked cone degeneration, it has been hypothesized that transsynaptic degeneration of ganglion cells can occur in cone dystrophies. Our histological observation of the atrophic changes in the ganglion cell layer confirm that transsynaptic degeneration does occur in association with cone degeneration. The mechanism by which a deletion in the red cone pigment gene affects ganglion cell structure is not known.

The immunohistochemical results show a significant diminution of the red and green cone population when compared with an age-matched control eye. Some remaining red and green cones without outer segments in the eye with X-linked cone degeneration did stain weakly with the antibody to red and green opsins in the inner segments. This contrasts with the strong staining pattern of the outer segments of the red and green cones in the control eye. We hypothesize that the remaining red and green cones have accumulated opsin in remaining cone inner segments (Figure 6, A) in contrast to the control eye, where this antibody strongly stained outer segments (Figure 6, C). In contrast, antibody specific for blue cone opsins similarly stained the outer segments of the patient and control eyes (Figure 6, B and D).
with death of cones. The reason for the pathologic changes in the retinal pigment epithelium also remains to be clarified.

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