A Histopathologic Study of a Choroideremia Carrier

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We have examined eyes from a heterozygote (carrier) of choroideremia, an X-linked disease. Gross examination revealed irregular pigmentation at the level of the retinal pigment epithelium (RPE) except at the posterior pole, and islands of well defined depigmentation of 1-4 mm in diameter in the midperiphery. The optic nerve and retinal blood vessels appeared normal, and there was minimal pigment migration into the retina. Histopathologic examination showed normal photoreceptors in the posterior and anterior fundus, but the outer segments were short or absent in much of the equatorial region. Little gliosis was noted in areas of retinal atrophy. The RPE was abnormal, with irregular thickness and pigmentation associated with variable lipofuscin content from one RPE cell to another, as shown by fluorescence microscopy. There were areas of profound atrophy in the equatorial region, with abrupt transitions between relatively normal RPE and photoreceptors, and retina devoid of RPE and photoreceptors. Bruch's membrane was thickened to a greater extent than is common in age-related change. The choriocapillaris was normal in areas with normal photoreceptors, except for widening of the intercapillary pillars. In those regions with abnormal photoreceptors, choriocapillaries were fewer in number, had reduced luminal diameter, and fenestrae were sparse. In some areas of intense atrophy, there were no choriocapillaries. The findings are compatible with the primary defect residing in the RPE. The Lyon hypothesis of X-chromosome inactivation and mosaicism could explain the irregularity of change and areas of intense atrophy, but abrupt demarcation between grossly abnormal, and relatively well preserved retina also occurs in hemizygotes (affected males). Invest Ophthalmol Vis Sci 31:229-236, 1990

Choroideremia has been considered a distinct nosologic entity since its original description.1 It is an X-linked disease,2 transmitted by a gene on the long arm of the X-chromosome.3-7 Like disorders within the category "retinitis pigmentosa", the atrophy starts in the midperiphery of the ocular fundus in affected males. However, unlike retinitis pigmentosa, atrophy of the retinal pigment epithelium (RPE) and inner choroid can be detected early in the course of the disorder. The inner retina is well preserved, as manifested by the normal appearance of the optic disk and retinal blood vessels; also, pigment migration into the neurosensory retina is not prominent. Histopathologic studies of eyes from males affected by choroideremia are few.8-12 These confirm the clinical impressions concerning the distribution of tissue loss. The RPE is diffusely abnormal in all cases, being absent in some areas and irregular in thickness and pigmentation elsewhere. The choriocapillaris is atrophic, while the inner retina is relatively well preserved. At sites of most profound atrophy in the midperiphery, there is absence of inner choroid and RPE, and the junction between normal and atrophic tissue is often sharp. Thickening and calcification of Bruch's membrane and preretinal fibrosis have been reported, although it is not clear whether these changes are integral to choroideremia or, alternatively, can be ascribed to age alone.

Females who are heterozygous for the abnormal gene show irregular pigmentation at the level of the retinal pigment epithelium, but only a small proportion have clinical symptoms.13 It is not uncommon for them to have detectable functional loss of vision in middle and late life. Severe visual deficit has been
recorded rarely, and in such cases the ocular fundi show well defined areas of atrophy in the equatorial region.\textsuperscript{13-15} Currently, only two reports, one of which is brief, exist concerning the histopathologic findings in heterozygotes.\textsuperscript{16,17} In heterozygotes, as in hemizygotes, the RPE was shown to be irregular in pigmentation and thickness, but nowhere was it absent. The photoreceptor cells were not reduced in number, but their outer segments appeared to be disorganized.

Various conclusions as to the cell system harboring the primary defect have been drawn from the histopathologic findings. It has been suggested that it may lie in the RPE,\textsuperscript{11} choroid,\textsuperscript{12} or in the metabolic relationship between RPE and photoreceptor cells.\textsuperscript{8}

We have had the opportunity to examine eyes from another heterozygote of choroideremia. Our findings differ in some respects from those of heterozygotes previously examined.

**Materials and Methods**

**Case History**

The donor was of Norwegian ancestry and was a demonstrated heterozygote for choroideremia. None of her ancestors was known to have significant eye disease. Her son had choroideremia, and the characteristic fundus changes of the heterozygote state had been recorded in the propositus, in one of her two daughters, and in all three daughters of her affected son (Fig. 1). The patient had loss of left eye central vision at the age of 76 yr due to a hemorrhage from a retinal macroaneurysm. The lesion was treated by laser photocoagulation, and her visual acuity recovered. At the time of her last eye examination, in 1986, visual acuity was 20/40 with each eye; the fundi showed reticular pigmentation at the level of the RPE; and there were early lens opacities. It was thought that the cataracts were sufficient to account for the loss of acuity. The patient died in November 1987, at the age of 78, of pulmonary infection 1 month after a stroke. Prior to this she had been well, except for mild heart failure, for which she had been treated with digoxin. She was receiving no other medication.

**Tissue Processing**

The eyes were enucleated 1 hr and 45 min after death, and the right eye, which is the subject of this study, was placed in a solution of 2.5\% glutaraldehyde and 2\% formaldehyde in 0.1M Na phosphate buffer at pH 7.2. The left eye was frozen and kept at -80°C for biochemical analysis. The anterior segment of the aldehyde-fixed eye was removed. Four narrow radial wedges extending from the macula to the ora serrata at 90° to one another were cut from the eyecup for initial microscopic survey. These were post-fixed in 1\% OsO\textsubscript{4}, dehydrated in ethanol, infiltrated in propylene oxide, and embedded in Araldite 502 epoxy resin (Ciba, Summit, NJ). After the survey, the remaining quadrants were photographed for recording purposes and were subdivided according to fundus characteristics; a total of 60 pieces of tissue were obtained. The specimens were divided into three groups, each representing one of the various parts of the fundus. One group was post-fixed in OsO\textsubscript{4}, infiltrated, and embedded in Araldite, as described above. The remaining tissue fragments were transferred to a storage solution of 2\% formaldehyde in 0.1\% Na phosphate buffer, pH 7.4, for 5 days at 4°C, to increase antigenicity.\textsuperscript{18} Half were then dehydrated in a graded series of ethanol and were embedded in London Resin (LR) White methacrylate resin for fluorescence microscopy and immunocytochemistry (Polysciences, Warrington, PA). The final group of specimens was embedded in diethylene glycol distearate for future in situ hybridization studies. As in a previous study,\textsuperscript{19} we followed the nomenclature of Duke-Elder\textsuperscript{20} and Hogan et al\textsuperscript{21} for the regional division of the ocular fundus.

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**Fig. 1.** Pedigree showing X-linked inheritance. The subject of this study, I/1, is a demonstrated heterozygote, since she has an affected son and a carrier daughter.
Light and Electron Microscopy

Light microscopy was performed on 1-μ sections from regions embedded in Araldite and were stained with toluidine blue. Photomicrographs were taken at magnifications of X25 and X40. Electron microscopy was performed on thin sections (400 Å) stained with uranyl acetate and lead citrate and examined with a JEOL 100C electron microscope. Fluorescence microscopy was performed on 1-μ sections of LR White-embedded retina with a Zeiss Photomicroscope III (Carl Zeiss GMBH, Oberkochen, West Germany) equipped with epifluorescence condenser III RS, excitation filters UG 51 and BG 3, and barrier filter LP 478.

Results

Gross Morphology

The results in this paper are limited to the morphologic changes seen in the patient's right eye. When the eye was opened, gross examination of the fundus revealed irregular pigmentation at the level of the RPE over the whole fundus, except at the posterior pole. This appeared as interconnecting lines of relative hyperpigmentation, most of which were radial (Fig. 2). In addition, there were islands of well defined depigmentation from 1-4 mm in diameter in the midperiphery of each quadrant. The optic nerve and retinal blood vessels appeared to be normal, and there was no evidence of pigment migration into the neurosensory retina. A hemorrhage within and deep to the retina was seen in relationship to the superior temporal retinal artery about 2 mm from its origin at the optic disc (Fig. 2). This was believed to be derived from a previously unsuspected macroaneurysm and was confirmed by histologic examination.

Light and Electron Microscopy

With the exception of regions of profound atrophy, the RPE was seen as a monolayer in most of the eye but in all areas was irregular in thickness and pigmentation (Fig. 3). Some cells were devoid of pigment, and others contained very heavy pigmentation. The nonpigmented cells were very thin, while the pigmented cells were of variable thickness, some being of normal height and others much taller than...
normal, with occasional RPE hyperplasia (Figs. 3–5). Fluorescence microscopy showed marked accumulation of autofluorescent particles, which we presume to be lipofuscin, in all pigmented RPE cells, even in areas where the outer segments were sparse (Fig. 3). The irregularity in RPE cell thickness appeared to be due to lipofuscin accumulation.

The photoreceptors of the foveal, parafoveal, and preequatorial retina appeared normal in number and length (Fig. 4). With progression towards the equator from posterior and anterior fundus, the photoreceptors became fewer in number and their outer segments became shorter or absent (Figs. 3, 5). The distribution of change was somewhat irregular; there were areas of few photoreceptors surrounded by regions with larger numbers. The density and length of the photoreceptors did not appear to correlate with the degree of RPE pigmentation.

At the equator there were few photoreceptors, and a minority had intact outer segments; however, we did see regions that approached normal appearance. In the postequatorial, equatorial, and preequatorial regions, there were isolated patches of profound atrophy of the outer retina which corresponded with the areas of depigmentation seen on gross examination. The transition from relatively normal RPE and photoreceptors with outer segments to retina devoid of RPE and outer retinal neurones was abrupt (Fig. 6). Over most of the atrophic areas there were no photoreceptor cells. In these cases the inner nuclear layer abutted Bruch's membrane. There was remarkably little gliosis in the atrophic retina. In the preequatorial retina there was microcystic degeneration, and around the microaneurysm there was intraretinal blood and preretinal fibrosis.

Bruch's membrane was thickened over most of the fundus, with the additional material deposited in a linear fashion both in the inner and outer collagenous layers (Figs. 3–6). The outer deposits intruded into the choroid, causing widening of the intercapillary pillars. Drusen were seen as distinct from the linear deposits in the posterior half of the fundus (Fig. 3), and limited subretinal neovascularization was seen in the preequatorial region.

The choriocapillaris was normal in areas with normal photoreceptor population, except for widening of the intercapillary pillars. In the regions in which the photoreceptors were abnormal, the choroidal capillaries were fewer in number and had reduced luminal diameter, and fenestrae were sparse. Few choroidal capillaries were seen in the areas of profound atrophy (Fig. 6).

Discussion

It is important in the evaluation of an inherited disorder in a patient of this age that normal aging changes be differentiated from those due to the inherited defect. The microcystoid degeneration and preretinal fibrosis at the site of the microaneurysm

![Fig. 4. Well preserved photoreceptors in the macular region with well ordered outer segments, intact choriocapillaris, and linear deposits of material within Bruch's membrane (A) (×2000). Anterior to the macula some disorganization of outer segments is seen with migration of a pigment-containing cell in the outer retina (B) (×1200).](image-url)
Fig. 5. (A) As the equator is approached there is progressive loss of photoreceptor outer segments and reduction of photoreceptor numbers. (B, C) The RPE is irregular in thickness, with lipofuscin accumulation in some cells. (A) X1800; (B, C) X4000.

can be ascribed to the effects of age and to hypertension. The changes in Bruch's membrane, the widening of the choroidal intercapillary pillars, and the accumulation of lipofuscin in the RPE cells are qualitatively similar to those seen in age-related macular disease, although in this case they were more widespread, more intense, and more irregular than is commonly seen with age alone. Similar characteristics were noted by Ghosh and McCulloch in a heterozygote. The current concepts of age-related macular disease ascribe the deposits within Bruch's membrane to defective breakdown of the contents of phago-}

somes within the RPE. It is believed that this breakdown results in the deposition of large molecules on the inner surface of Bruch's membrane, which accumulate because they do not diffuse towards the choroid. If these concepts are correct, they imply previous active outer segment renewal and shedding, as well as RPE autophagy in the areas with Bruch's membrane deposits and large quantities of lipofuscin in the RPE. The persistence of these deposits in regions with few photoreceptors and no outer segments is surprising, since it is believed that there is constant turnover of this material. This accumulation could be explained by inability to clear the debris, due to choroidal capillary closure.

The cellular changes in the eye that can be attributed to the phenotypic expression of the choroideremia gene are in accord with many of the previous descriptions of choroideremia in affected males and heterozygous females. The photoreceptors were normal only in the anterior and posterior retina. In every region, the RPE appeared abnormal, even in areas in which the photoreceptor cells were within normal limits of population density and outer segment length. The choriocapillaris was normal only in those areas with normal photoreceptor density. The severity of affection in this eye was greater than has been recorded in previous histopathologic studies of heterozygotes. However, the extent of cell loss is not surprising, given the severity of visual loss recorded in some patients.

The regional distribution of abnormality with relative preservation of the central and peripheral retina is also in agreement with the pattern of cell loss which occurs in hemizygous males, although the severity of change in this latter case was variable within each region. Superimposed upon this pattern were very well defined isolated areas in which the photoreceptors, RPE, and choroidal capillaries were absent. No explanation exists for the predilection of the disorder to start in the midperiphery, or for the changes to be most severe in this region, although this is a characteristic of many of the general retinal dystrophies. It is tempting to invoke the Lyon hypothesis to explain the regional variability and the areas of intense atrophy; according to this hypothesis, the phenotype of the abnormal area would be determined by the abnormal gene, while in the relatively normal surrounding area the chromosome with the abnormal gene would have been inactivated. However, the abrupt demarcation between grossly abnormal and relatively well preserved retina is also found in hemizygotes, which implies that this demarcation may be a characteristic integral to the disorder rather than a manifestation of the heterozygous state.

There is no clear indication as to which cell system
harbors the primary defect. The integrity of the RPE suggested to Ghosh and colleagues that the RPE alone was not abnormal, but rather that the disease mechanism involved abnormal interaction between the photoreceptors and RPE cells. Cameron and co-workers observed that the initial morphologic changes were seen in the choroidal capillaries; they acknowledged that it would be more attractive, how-
ever, to implicate the RPE. The most persuasive current evidence concerning the cellular location of the initiation of the disorder is the identification of abnormally high levels of cyclic AMP in the RPE and choroid by Rodrigues and colleagues. 11 They concluded from this evidence the RPE was the likeliest site of the primary defect. However, our analysis of cAMP levels in the RPE and choroid of the current case showed abnormally high values in only 2 of 87 samples analyzed, with low levels in some areas and normal levels in others. 30

In summary, the distribution of change in the heterozygote of the current study differs from that seen in most histopathologic studies of retinitis pigmentosa, in which the photoreceptors show the most intense changes, the RPE usually is continuous, and atrophy of the choriocapillaris occurs in only the most advanced disease. 11 These findings can be contrasted with the discontinuity of the RPE and marked changes in the choroid seen in the current case. Our findings and those of other studies are compatible with initiation of the disorder by the RPE, although they do not represent prima facie evidence in support of this concept. The abnormal appearance of the RPE throughout the eye in this study, even in areas of normal choroid and neurosensory-retina, supports this conclusion. Since the variability in lipofuscin accumulation was not correlated with photoreceptor population, it may be related to some attribute of RPE dysfunction. That there was evidence of photoreceptor loss in areas with intact RPE does not exclude the possibility that the RPE plays a role. It is quite conceivable that a primary defect in the RPE may cause photoreceptor cell death without RPE cell death. Changes in the choroid also may be due to RPE dysfunction. There is good evidence for metabolic interaction between the RPE and choriocapillaris, 12,33 and it is possible that the normal morphology and functional characteristics of the choroidal capillaries are determined by diffusible factors produced by the RPE. 34 It is hoped that more precise evidence as to the nature and location of the primary disorder will be derived from additional studies on this eye.

Key words: choroideremia, photoreceptors, pigment epithelium, Lyon hypothesis, retinal degeneration

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References


