Image Analysis of the Tapetal-Like Reflex in Carriers of X-Linked Retinitis Pigmentosa

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Purpose. To increase understanding of the tapetal-like reflex (TLR), a unique retinal feature in carriers of X-linked retinitis pigmentosa (XLRP).

Methods. Color fundus photographs of XLRP carriers were digitized at high resolution. A mathematical model of the imaging system was used to restore the digital retinal images. TLR was separated from the retinal background with an automated segmentation method. Mathematical morphology was used to estimate directional properties. Images from serial photos were registered and compared to study temporal progression.

Results. Quantitative analysis of well-focused funduscopic images show point-like unit reflexes forming the TLR. The average unit reflex is circularly symmetric with a diameter of approximately 8.5 μm and has a maximum reflectance 40% higher than that of the neighboring nonreflex retina. Two or more unit reflexes form small elongate patches that can cluster together into larger patches. Both smaller and larger patches have a strong preferential direction toward the fovea. Comparison of images taken 23 years apart in one patient and 3 years apart in another patient show no detectable changes in the size and location of the reflexes.

Conclusions. The pattern of reflexes at high and low resolution suggests that the TLR represents an X-inactivation mosaic. Based on the size of the unit reflexes, the authors speculate that the cone photoreceptors participate in the TLR. The stability of the reflex over more than two decades questions the longstanding assumption that the TLR is a stage of the retinal degeneration. Invest Ophthalmol Vis Sci. 1994;35:3812-3824.
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gres,\textsuperscript{10,11} were used in this study; photographs from both eyes were available in all patients except P5. The ages at the time of the photography for P1, P2, and P3 were 17, 36, and 42 years, respectively. Two patients were photographed twice: P4 at 21 and 44 years and P5 at 48 and 51 years of age. Eight of the nine eyes used in this study had a spherical equivalent refraction of less than $-1.75$ diopters; maximum astigmatism was $+1.00$ diopters. Refraction in the right eye of P1 was $-6.25 + 5.50 \times 10^5$. All subjects gave their informed consent after the nature of the procedure had been explained. The research was in accordance with institutional guidelines and with the Declaration of Helsinki.

**Imaging System**

Fundus photographs were taken with a 30° camera (Zeiss, Oberkochen, Germany) and a flash energy setting of 60 Watt-seconds (Ws). In P5, 15° fields were also obtained using a 2X attachment and a flash energy setting of 120 Ws. All images were recorded on 100 ASA transparency (slide) film (Fujichrome; Fuji Photo Film, Tokyo, Japan), except for the photographs of P4 when she was 21 years old, which were recorded on 64 ASA film (Kodachrome; Eastman Kodak, Rochester, NY).

The fundus photographs were digitized using a scanner (LS3500, Nikon, Tokyo, Japan) at its highest spatial resolution. At this resolution, a standard 35-mm film can be digitized into $6144 \times 4096$ pixels with a pixel size of $6 \times 6 \mu m$ measured on the film. The intensity of each pixel was quantized to 256 levels. The ability to control the integration time used during scanning allowed us to select the optical density range to be mapped to the 256 levels. For example, pixel values in the range of 0 to 255 corresponded (nonlinearly) to film optical densities of 2.0 to 1.0 at the longest integration time, respectively, and 2.0 to 0.1 at the shortest integration time, respectively. Intensity values of each pixel were compensated for bias and gain differences that existed among the detectors of the scanner.

The dimensions of a pixel corresponded to 0.50 minutes of arc (0.50°) and 0.25 minutes of arc (0.25°) for the 30° and 15° field of view photographs, respectively. Assuming emmetropia and a linear magnification of 2.5 for the Zeiss 30° camera, the approximate pixel size measured on the retinal surface was $2.4 \times 2.4 \mu m$ for the 30° field of view and $1.2 \times 1.2 \mu m$ for the 15° field of view. The actual dimension of a pixel on the retina depends on many factors, including objective lens to eye distance, camera back to objective lens distance, refraction, globe length, and the retinal location of the pixel.\textsuperscript{10,12}

The scanner is capable of digitizing a color transparency into red, green, and blue components by using filters between the scanning light and the film. Preliminary results showed that the green component of the TLR images produced the highest contrast. In this study, “green” images were analyzed unless otherwise noted. The information content of the green image effectively represented that of a light detector with an approximate peak sensitivity of 550 nm and an approximate bandwidth (full width at half-peak sensitivity) of 50 nm; that of the “red” image had a peak sensitivity of 650 nm and a bandwidth of 30 nm.

**Image Processing**

The characteristics of the TLR that were studied are related to the spatial variation of light intensity reflected from the fundus. The magnitude of the reflected light is not directly available in fundus photography because the recorded information is the optical density of color film; i.e., three types of dyes with relatively broad spectral sensitivities. Therefore, an attempt at quantitative analysis of the TLR requires the estimation of the light intensity that originated from the fundus based on the optical density image recorded on film. For this purpose, a mathematical model was developed based on four components of the imaging system: the eye, the camera, the film, and the scanner. The eye and the camera were each modeled as optical systems that blur the spatial distribution of light without adding noise. The extent of blurring produced by each system was defined by isotropic transfer functions. The transfer function of the eye was taken as that of an average eye with a 1.5-mm pupil.\textsuperscript{14} The transfer function of the camera was approximated with a first-order exponential decay function dropping to 10% modulation at 100 cgc/mm measured on the fundus. The film model consisted of three parts. The first part blurred the spatial distribution of light and was based on an isotropic transfer function. The second part related the resulting film density to the input light intensity through a nonlinear transfer function, and the third part of the film model described the characteristics of the additive random noise, the parameters of which varied as a nonlinear function of mean film density.\textsuperscript{15,16} All parameters modeling the film were taken to be average values reported by the manufacturer. The last component of the imaging system, the scanner, was also modeled with three parts. The first part related the film density to light intensity through a nonlinear function. The second part blurred the spatial distribution of light based on an isotropic transfer function, and the third part described the characteristics of the additive random noise, the parameters of which varied as a function of mean light intensity.\textsuperscript{15} The transfer function, the point nonlinearity, and the noise parameters of the scanner were measured experimentally. Details of the imaging
system model and its validation can be found elsewhere. 18,19

The imaging system model allows the estimation of the digital image based on the knowledge of the light intensity originating from the fundus. In this work, the opposite is necessary: the estimation of the light intensity that originated from the fundus based on the known digital image. For this purpose, a digital image restoration method was developed that attempted to invert the nonlinearities introduced by the film and the scanner, to reduce the image noise, and to recover the diminished high-frequency information. The complete nonlinear imaging system model was first simplified to a more mathematically tractable “generalized linear model,” which consisted of a single equivalent blurring function and a single equivalent additive noise source enclosed with point nonlinearities. This simplification made it possible to use regularized linear image restoration, 20,21 a classic method that finds the best compromise between enhancing intermediate spatial frequency information and suppressing the high spatial frequency noise. Details of the restoration method and the validation of its results have been published. 19

A method called multiscale segmentation was developed to automate the detection of the numerous TLR patches in each region studied. 19,22 The method classified each pixel as “object” or “background” based on consideration of the intensities of pixels within two differently sized neighborhoods. This segmentation method permitted good separation of distinct objects as well as a low rate of falsely detected objects. Details of this method and its performance on simulated images and TLR images have been published. 19,22

A registration method was developed to assess temporal progression of the TLR. 19,23 The method assumes that one of the images to be registered is a translated, rotated, and uniformly scaled version of the other; that is, four parameters, x-axis translation, y-axis translation, rotation angle, and scale factor, define the transformation to be applied to one image to bring it into alignment with the other image. The registration method uses the cross-correlation function of invariant descriptors to determine the optimum set of parameters in a four-dimensional discrete parameter space. To refine the resolution of the parameters thus calculated, a limited search in the four-dimensional continuous parameter space is applied in the neighborhood of the already calculated coarse parameters. The method has been shown to achieve high registration accuracy; its details and performance have been described elsewhere. 19,23

Analysis of the directionality of TLR patches was accomplished using mathematical morphology. 24,25 A method called directional morphology was developed for this purpose. 19,26 Segmented TLR images were morphologically filtered with progressively longer line-shaped structuring elements with a sequence of orientations. The orientations were chosen in 10° increments from −90° to +90°, and the orientation at each pixel location was normalized by the orientation of the fovea. Thus, the orientation of 0° anywhere in an image corresponded to the orientation of a line connecting that pixel with the fovea, defined as the geometric center of the macular region. Details and validation of this method are given elsewhere. 19,26

RESULTS

Restoration and Segmentation of the Images

Figure 1A shows a 30° field of view photograph of the left eye of P1. The TLR appears most dense and bright in the temporal perifoveal retina and is not visible near the fovea. Details of image restoration and segmentation are shown (Figs. 1B, 1C, 1D) for a 2° X 2° region of the photograph (marked by the white square in Fig. 1A). The effects of noise are clearly visible in the enlarged version of this 256 X 256 pixel region (Fig. 1B). The result of digitally restoring the region (Fig. 1C) is to reduce the image noise and simultaneously minimize the blurring of features. It is important to note that the result of the restoration process is an estimate of the original fundus luminance; that is, values of pixels linearly relate to the amount of light reflected from the fundus, because imaging system nonlinearities have been (partially) inverted.

The application of the automated image segmentation method is shown in Figure 1D, where white pixels correspond to the reflex, and black pixels correspond to the retinal background. In this context, segmentation may be viewed as a “feature extraction” process, in which estimated luminance values calculated for each pixel during restoration are converted into one of two classes: reflex or background.

Interocular and Intraocular Variation of Density

In Figure 2, the methods described in Figure 1 are used to make comparisons of TLR density in different regions at comparable eccentricities in the same eye and between regions in the two eyes of four heterozygotes. The photographs in Figure 2 are from the right and left eyes of P2. White squares (5° X 5°) mark the three regions studied (temporal, superotemporal, and inferotemporal); all three regions were at an eccentricity of approximately 12° (measured from the geometric center of the fovea to the center of each square). Each 640 X 640 pixel region was restored and segmented, and the fraction of pixels classified as...
FIGURE 1. (A) Fundus image of the left eye of P1 showing the TLR. A subregion is outlined with a white square. (B) Magnified version of the subregion. (C) Result of digital restoration applied to (B). (D) Result of automated segmentation applied to the restored image in (C). White pixels correspond to TLR, and black pixels correspond to background.

“reflex” in the segmentation results are shown as bar plots in Figure 2.

There was a range of density from 0.05 to 0.28 in the regions sampled. Some eyes showed little meridional variation in TLR density (e.g., left eye of P1; right eye of P3), but most eyes showed considerable variation. All four patients had interocular asymmetry in TLR density in at least one of the three regions sampled; some examples of similar densities in the two eyes were present (e.g., temporal region, P1 and P4; superotemporal region, P3 and P4). The results in these heterozygotes indicate that there can be both interocular and intraocular variation in the density of the TLR.

Size and Brightness Properties

On visual inspection of 30° field of view fundus photographs, it was noted that there were occasional bright, dot-like, individual reflexes. Such reflexes appeared at the same location on multiple fundus photographs, eliminating the possibility that this was random noise due to individual film grains. We hypothesized that blurred aggregates of these dot-like reflexes form the larger patches making up the TLR, and at the magnification of the 30° field of view photographs, the individual dot-like reflexes are not commonly discernible. The best-focused of a set of photographs with a 15° field of view taken of P5 showed that, in many instances, the TLR did consist of clusters of tiny dot-like structures, which we will refer to as “unit” reflexes.

Figure 3A shows the TLR in the right eye of P5. Two 2.5° × 2.5° regions (5° and 13° temporal to the fovea; white squares) with especially high density of discernible unit reflexes were selected for analyses of the unit reflex size and brightness properties. Figure 3b shows a magnified view of the more central region, and the white circles highlight clusters of distinct unit reflexes. Hundreds of such unit reflexes (n = 491 and n = 358 at the 5° and 13° regions, respectively) were specified manually in both regions. The pixel values...
on a horizontal line (horizontal profile) and a vertical line (vertical profile) passing through the peak of each reflex were determined. Each profile was normalized by the lowest intensity within 10 pixels of the peak. All normalized profiles for a region were then averaged. Image restoration was not used in this analysis, to minimize any potential artifacts due to changes in the spatial frequency content of the images. Only static nonlinearities due to the scanner and the film were inverted.

Mean vertical profiles for the two regions are shown in Figure 3c. The horizontal profiles displayed comparable results. Profiles applied to the red image showed a similarly shaped curve for both regions with reduced peak contrast (data not shown). Profiles from blue images were not obtainable because of the extremely low signal-to-noise ratio, probably due to the known low reflectance of the fundus in the blue region. The data from the high magnification fundus photograph of P6 thus suggest that the TLR is made of unit reflexes.

The contrast between the peak reflectance within a unit reflex and its immediate nonreflex background and the spectral dependence of the contrast were determined. The peak reflectance in the green region of the spectrum is approximately 1.4 times that of the neighboring nonreflex retina, whereas this ratio is approximately 1.25 in the red region. The extent of the unit reflexes (full width at half maximum of average profile) is no greater than 0.03°.
(1.75°, approximately 8.5 μm) vertically, and the extent is similar in the horizontal direction. The size and reflectance properties of the reflexes were the same at the two eccentricities analyzed in the temporal retina of P5. It should be considered that the unit reflex size may be an overestimate and the peak reflectance an underestimate because of blurring in the imaging system.

Directional Properties

The 30° field of view images in Figures 1 to 3 convey the impression that the TLR radiates from the fovea toward the periphery. Macroscopically, there are regions of higher and lower reflex density that form radial sectors or zones centered at the fovea. Figures 4 and 5 explore quantitatively the directional tendencies of the TLR at higher resolution. Figure 4A shows a magnified view of a 2.5° × 2.5° region selected from the right eye of P3. The 320 × 320 pixel region was restored and segmented (as in Fig. 1D), and the outlines of the segmentation results are shown in Figures 4B, 4C, and 4D. Directional morphology with a bar-shaped probing element was applied to the segmented result. The length of the probing element was 41 pixels (0.9°, approximately 100 μm). Figure 4B shows the subset of pixels that were compatible with the probing element oriented toward the fovea. The subsets of pixels compatible with the probing element oriented 60° away in either direction from the fovea are shown in Figures 4C and 4D. It is evident that the directional tendency in this small region is toward the fovea.

Figure 4E shows 17 regions that were processed for directionality throughout the posterior pole of the right eye of P3. An ellipse is superimposed on the center of each region analyzed. The orientation of the long axis of each ellipse is the peak of the directional morphology result (i.e., the fraction of segmented pixels found to be compatible with the probing element as a function of its rotation angle). All 17 regions showed highly directional results; most of them had zero pixels orthogonal to the peak direction. The peak direction, however, was not always oriented exactly toward the fovea; 13 regions had peak direction within 10°, and four were within 30° of the fovea.
The 15° field of view, high-resolution photograph of the right eye of P5 was used to analyze the directionality of small elongated patches (Fig. 5). Each 2.5° × 2.5° (640 × 640 pixel) region was restored, segmented, and analyzed with directional morphology methods. The probing element used for directional morphology was a 4.8'-long bar (19 pixels, approximately 23 μm) rotated to all directions at 10° increments. The directionality results were more variable than those at the medium resolution level. The ratio of the number of pixels in the peak direction to the number of pixels in the orthogonal direction varied from 8 to 1.5. Furthermore, the fraction of segmented pixels found to be in the peak direction also varied from 0.6 to 0.3. The ellipses representing the directionality results in Figure 5 code three parameters: the orientation of the long axis is the peak direction (as in Fig. 4E); the length of the long axis is proportional to the fraction of segmented pixels found in the peak direction; and the ratio of long to short axes is equal to the ratio of the pixels in the peak direction and in the orthogonal direction.

The majority of the regions analyzed (26 of 34) in Figure 5 showed strong directional tendencies oriented within 20° of the direction toward the fovea. Among the eight remaining regions, four did not show high directionality (low peak-direction-to-orthogonal-direction ratio). The other four regions were highly directional, but their peak orientation was not toward the fovea. The possibility that the calculated direction was an artefact of nonisotropic blurring during the scanning process was ruled out by scanning the same region in two orthogonal directions.
FIGURE 5. Fundus image from the right eye of P5. White squares outline 34 subregions analyzed at high resolution with directional morphology using a bar-shaped probing element of 19 pixels. Overlaid ellipses summarize the results: The direction of the long axis is the peak direction; the ratio of long to short axis lengths equals the ratio of pixels in the peak direction to pixels orthogonal to the peak direction; and the length of the long axis is proportional to the fraction of segmented pixels in the peak direction.

Temporal Properties

The temporal progression of the spatial distribution of TLR was studied in P4 and P5. Changes in absolute intensity of the reflex would be difficult to measure, considering the variability of photographs taken years apart (due to effects of fundus illumination, focus, film type and development, and effects of film storage).

Figure 6 shows photographs of the left eye of P4 and the two 4.3° × 4.3° (640 × 640 pixels; white squares) regions selected for temporal comparison. Subregions of 512 × 512 pixels were cropped after registration and transformation and are also displayed in Figure 6. The scanned images had qualitative differences in brightness, the basis for which is uncertain. Whether these differences were due to the different film characteristics, the effects of 23 years of storage, or inherent differences in the fundal reflectance cannot be determined. Therefore, the digital images are shown arbitrarily and independently scaled for best qualitative contrast. Small triangles mark the line where the pixel values from both images are compared in graphical form below. The results show that the location and the size of the particles making up the TLR have not changed substantially over 23 years in this patient. The same results were obtained in the other eye of P4 and in a comparison of photographs at a 3-year interval in P5.

DISCUSSION

The present study represents the first attempt to analyze quantitatively the TLR, a unique retinal reflection found in some heterozygotes of XLRP. We applied digital image processing methods to fundus photographs of the TLR and studied its morphologic, radiometric, and temporal properties. In summary, the TLR appeared macroscopically to be made up of radiating sectors or zones, but microscopically it was composed of small round unit reflexes, the average diameter of which was approximately 8.5 μm. The peak reflectance of the unit reflexes was 40% greater than
that of nonreflex retina at 550 nm and 25% greater at 650 nm. There was intraocular and interocular variability in spatial density of the TLR, but there was consistency in the directional preference of TLR patches toward the fovea in all eyes studied. Serial photographs at 3-year and 23-year intervals showed no detectable changes in the size or location of the TLR patches, although time-dependent changes in the brightness of the reflex could not be quantified with current methods. Based on these observations, we attempt to answer questions about the TLR and X inactivation, the origin of the reflex, and the relationship of the TLR to retinal degeneration in XLRP heterozygotes.

Tapetal-Like Reflex and X Inactivation
The association of the TLR with the heterozygous state of XLRP leads to the question of whether TLR is an...
ophthalmoscopically visible representation of the X-inactivation mosaic. In brief review, the X-inactivation hypothesis states that one of the two X chromosomes (paternal or maternal in origin) in each cell of a female is randomly inactivated early in development, and once this event has occurred, it is fixed within the somatic heredity of the cell.29-31 The inactivation causes tissues to form a mosaic of two populations of cells, which are subjected to forces such as growth, mingling, migration, and selection that lead to modifications of the mosaic.32-34

Classic examples of X inactivation are the murine X-linked coat color mutations, the variegated coat color representing large patches presumably due to early inactivation and clonal growth.39,40 However, examination of planar mosaics, such as the retinal pigment epithelium (RPE), have shown that patches as small as one cell can be found in X-inactivation mosaics.35,36 Experiments in chimeras with different photoreceptor and RPE genotypes also indicate that in the resulting mosaic, patch size can be small.37,38

Our results show sufficient similarities between the TLR and other X-inactivation mosaics to suggest that they are directly related. For example, the high resolution images of the TLR have a pattern (see Fig. 3b) that is reminiscent of previously described mosaics with a small patch size.35,39,40 Radiating sectors or zones seen macroscopically in the TLR have also been described previously in retinas of mouse chimeras and X-inactivation mosaics.36-41 These macroscopic sectors and the microscopic directional preferential orientation of TLR patches could be the result of the complex effects of ocular growth and development on the mosaic.42-44 Our finding of interocular asymmetry of TLR density is also consistent with the notion that the TLR is an X-inactivation mosaic. Recent experiments in mice indicate that X inactivation is tissue specific and that it occurs later than lateralization of the optic vesicles.45 If random inactivation occurred independently in each eye of XLRP heterozygotes, interocular asymmetry in the mosaic of each eye would be expected.

Origin of the Tapetal-Like Reflex

Ophthalmoscopists have described the TLR as lying deep in the retinal blood vessels and at the level of the outer retina and RPE.4,6,46-49 Fluorescein angiography has not shown abnormalities in the region of the reflex.48,50 It has been suggested that the TLR could be the result of deposits, thickening, or degeneration in Bruch’s membrane;5,51 deposits in the retina;46,47; or an alteration at the level of RPE-photoreceptor interface.48 The presence of fundus reflexes resembling the TLR in retinopathies with cone dystrophy or dysfunction led to the suggestion that the TLR indicated involvement of the cone photoreceptors.52 Morphologic studies of postmortem donor retinas from XLRP heterozygotes have not revealed any findings that clearly explain the TLR; these donor retinas, however, have not been from heterozygotes known to have a TLR5,5 (and Milam, unpublished observations).

Theories about the origin of the TLR are dependent on present on noninvasive investigations such as were performed in the present study. Our results could be used in support of many different theories about the origin of the TLR, but we will limit speculation to a single hypothesis. Assuming that the TLR represents the underlying X-inactivation mosaic, that it originates near the photoreceptor–RPE complex, and that the unit reflexes have an intracellular basis, we speculate that cone photoreceptors are the cells involved in the TLR. This notion derives mainly from our high-resolution data that showed unit reflexes to be circularly symmetric with a diameter of approximately 8.5 µm. Larger reflex patches seen ophthalmoscopically would represent blurred aggregates of unit reflexes. Average diameters of cone inner segments are 8 µm, whereas the diameters of rod inner segments and RPE cells are 2 µm and 15 µm, respectively, in the perifoveal regions, where our measurements were made.44,53,54 The temporal stability of the TLR would seem to favor the cone inner segment, rather than the renewing outer segment, as a possible site of the reflectance.

It has been reported that the TLR is not present at the fovea.4,6,46 This observation would appear to be inconsistent with the hypothesis that the TLR originates in the cone. Interestingly, visual inspection of images from P5 showed that some reflex was present in the region of the fovea; unfortunately, this region could not be analyzed with digital image processing because of the extremely low contrast. The altered visibility of the reflex at the fovea may be due to secondary effects such as the reduced cone inner segment diameter44 and filtering by the macular pigment.

The nature of the abnormality in the cone inner segment leading to the reflectance is also highly speculative. Because inclusion bodies have been identified in the inner segment of cones in normal and retinitis pigmentosa retinas,55,55-57 it is conceivable that intracellular particles with high refractive index are present in the cone inner segments of XLRP heterozygotes. Such particles could produce either specular or diffuse reflections, depending on their size, shape and arrangement.58 It is of interest that intracellular lipid droplets, fibrils, and crystals have been determined to be the reflecting materials in some types of tapeta in animals.7,58 It is also conceivable that an abnormal arrangement of normal cell components (for example, an abnormal gradient of mitochondria concentration) causes reflections.59 Of course, the cones could be passive participants in the TLR and be acting as light guides for more distal reflections.
That the reflex originates in the cone receptor would be in agreement with other data that indicate that cones have greater involvement than rod cells in certain patients with XLRP. The more mildly affected hemi- and heterozygotes of a large XLRP pedigree that we previously studied with retinal function tests showed more cone than rod photoreceptor dysfunction; P2 and P3 in the present study are from this pedigree. A young hemizygote in this pedigree, interestingly, showed a diffuse golden reflex across the retina and had only cone dysfunction. Assuming that the golden reflex in XLRP hemizygoties has the same basis as the TLR in heterozygotes, the diffuse nature of the reflex would be consistent with the lack of X inactivation. Golden reflexes have also been reported in patients with cone and cone–rod dystrophies.

**Tapetal-Like Reflex and Retinal Degeneration**

The TLR is one of two ophthalmoscopically detectable abnormalities in heterozygotes of XLRP, the other being patches of pigmentary retinopathy. In some heterozygotes, the two features are found in the same eye, whereas in others they occur independently. Donor retinas from heterozygotes with pigmentary retinopathy show variably sized patches of rod and cone photoreceptor degeneration (and Milam, unpublished observations), and the type of histopathology can resemble that of hemizygoties. These findings and other data have led to the suggestion that the pigmentary retinal degeneration resembles an X-inactivation mosaic. Furthermore, abnormalities in retinal function that reflect the underlying patchy retinal degeneration show interocular asymmetry, suggesting that random inactivation occurred independently in each eye of the heterozygotes.

Although the TLR and the patchy pigmentary retinopathy are different ophthalmoscopically, it has been assumed in the literature that the TLR represents an early stage of retinal degeneration. This is based mainly on some reports of fading of the TLR with time and the presence of a golden reflex preceding retinal degeneration in some young XLRP hemizygoties. Changes in reflex intensity with time were not quantified in the present work. The lack of detectable change in location and size of the reflexes over a 23 year interval in P4, however, suggests that, if a degenerative process is associated with the TLR, then it must be an exceedingly slow one. An alternative hypothesis is that the TLR and the patchy retinopathy are independent phenotypic expressions of the same gene abnormality. For example, the mutant gene product could be expressed in both photoreceptors and RPE leading to two different X-inactivation mosaics.

**Future Studies**

In the absence of histopathology, future progress in understanding the TLR may depend on further noninvasive studies. The retinal layer of origin of the TLR may be able to be estimated using techniques such as confocal laser scanning tomography, optical coherence tomography, and laser biomicroscopy. Whether small patches of TLR are associated with photoreceptor-mediated dysfunction may be amenable to study with methods such as fine matrix perimeter. The eventual elucidation of the molecular bases of XLRP and of the method by which the mutant genes lead to disease expression in hemizygoties should increase our understanding of the different phenotypic expressions in heterozygotes.

**Key Words**

heterozygote, digital image analysis, X-linked retinitis pigmentosa, tapetal-like reflex, X chromosome inactivation

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