Selective Cone Dystrophy With Protan Genotype

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Purpose. To determine the functional defects in two male patients with progressive cone dystrophy and hybrid L–M cone pigment genes.

Methods. Clinical evaluation, standard electroretinography, and electrooculography were performed in two affected patients and two family members. Measurements of spectral sensitivity and transient tritanopia were made in both patients.

Results. In the patients, visual acuity varied between 20/50 and 20/100. The electroretinogram showed reduced flicker responses. When light adapted, a-wave amplitudes were borderline, but b-wave amplitudes were reduced severely. Electroretinography with chromatic stimuli showed a difference between well-preserved responses to green and markedly reduced responses to red stimuli. Spectral sensitivity measurement revealed a lack of L (long-wavelength sensitive; red) cone function and normal function of the S (short-wavelength sensitive; blue) and M (middle-wavelength sensitive; green) cones. Transient tritanopia was abnormal, indicating a severe disturbance of cone–cone interaction.

Conclusions. Progressive cone dystrophy with predominant dysfunction of L cones exists in both patients. The cone dystrophy may be caused by a rearrangement of the X-chromosome pigment gene array that is associated with the deletion of L-cone sequences and the formation of hybrid L–M cone pigment genes. It cannot be excluded, however, that both patients have protanopia and that cone dystrophy developed because of other causes. Invest Ophthalmol Vis Sci. 1995;36:2381–2387.

Progressive cone dystrophies usually affect the spectrally different types of cones to the same extent. Common findings are reduced visual acuity, color vision defects up to total achromatopsia, and reduced or missing electroretinographic responses during light adaptation and 30-Hz flicker stimulation.[1-3] A predominant involvement of L cones in progressive cone dystrophies has been described recently.[4,5] Several combinations of selective impairment of one or two cone systems have been found in nonprogressive, incomplete achromatopsia.[6,7]

In retinal diseases, the a- and b-waves of the electroretinogram show specific alterations according to the affected retinal layers. Normal a-wave amplitudes and b-wave amplitude reductions are common findings in several retinal diseases involving the inner retinal layers or with defective neuronal transmission, e.g., in central retinal arterial occlusion, X-linked congenital retinoschisis,[8] or congenital stationary night blindness.[9] Cone dystrophies with normal or mildly reduced cone a-wave amplitudes and markedly reduced cone b-wave amplitudes are rare.[10-13]

We observed two unrelated males with progressive cone dystrophy with predominant L-cone dysfunction and severely reduced b-waves in the light-adapted recordings. Family members of one patient showed similar alterations of the electrophysiologic findings without clinical signs.

METHODS

In performing the examinations, the tenets of the Declaration of Helsinki were followed, informed consent was obtained, and approval was given by the institutional human experimentation committee.

Electrophysiologic Methods

Electrooculograms were recorded according to the method of Rhode et al.[14] The recording method for electroretinography has been described in detail.[8] The recording protocol included all recordings according to the standard for clinical electroretinography.[15] Stimulus duration was 10 msec. Six different light intensities (1 to 6) increasing stepwise by 1 logarithmic unit from the b-wave threshold of the normal eye were used for recordings in the dark-adapted state. The maximum light intensity was 7.8 cd·s/m². Light-adapted recordings were performed in the presence of white light adaptation of 4.5 cd/m² and with the light stimuli at 4 to 6. The 30-Hz flicker stimulus was 5 log units above threshold. Light from a xenon arc source served as the stimulus in all examinations.

In addition to the recordings with white stimuli, we used Kodak Wratten filters (Eastman Kodak, Rochester, NY) to produce chromatic stimuli (color electroretinogram[16]). The filters were Kodak Wratten #98 for blue with a maximum transmission at 450 nm, #44A for blue–green (492 nm), #61 for green (538 nm), #16 for yellow (589 nm), and #29 for red (629 nm). A white background was used for light adaptation. Recordings were made according to the protocol used for white stimuli. Except for the oscillatory potentials (64 sweeps), no averaging was made in recording the standard or color electroretinograms.

Psychophysic Methods

Dark Adaptation. Dark adaptation was tested with the Goldmann Weekers adaptometer. Sensitivity thresholds were determined after a 10-minute bleach of 1,400 cd/m² in a Ganzfeld during a 45-minute period. The target size was 11° in the diameter in the upper field 10° from the fovea.

Spectral Sensitivity. Spectral sensitivity functions were determined for 30 monochromatic test lights (13° in diameter, foveal fixation) in the presence of a white background (10,000 td, 30° in diameter). The quantal energy necessary for a threshold response evoked by different monochromatic test lights was plotted against wavelength. The sensitivity of S, M, and L cones was examined separately, suppressing the other cone systems by means of chromatic adaptation (489 nm for L cones, 422 + 650 nm for M cones, and 575 nm for S cones).

Transient Tritanopia. The quantal energy, necessary to evoke a threshold response for a blue (440 nm) test light of 13° in diameter, is determined at various times before (values <0) and after (values >0) switching off a yellow adaptation light (575 nm, approximately 10,000 td) of 30° in diameter.[17,18] In normals, the threshold to detect the blue test light raises immediately after the offset (delay = 0) of the yellow adaptation light for approximately 1 logarithmic unit and reaches the lower threshold that was obtained during yellow adaptation only 1 or 2 seconds thereafter.

RESULTS

Clinical Findings

Patient 1. The first patient was 43 years old at the time of electrophysiologic and psychophysic examinations. His general medical history was unremarkable. A reduction of visual acuity was known since he was 3 years of age, when glasses were first prescribed because of myopia. However, visual acuity was sufficient for him to obtain a drivers license at 18 years of age. Difficulties with color vision had been noted since childhood. At 42 years of age, deterioration of visual acuity, progressive color vision defects, and photophobia were noticed. The refractive error was −7.5 dpt on both eyes. Visual acuity was 20/100 on both eyes with contact lenses. Visual acuity increased subjectively with blue-filter glasses. Discrete horizontal nystagmus was not noticed until the most recent examination. Ophthalmoscopy showed myopic fundus changes, pigment irregularities within the macular area, and pale temporal optic discs on both eyes. Fixation was temporal superior to the fovea on both eyes. The visual fields tested with the Tübingen Perimeter showed enlarged blind spots and central scotomas within the 5° isopter.

The patient's mother had myopia (approximately −5.0 dpt), as did her brother, sister, and father. Further details were unavailable, and examination of family members was not possible because of old age or death. There was no history of cone dystrophy.

Patient 2. The second patient was examined on three occasions. His general medical history was unremarkable except for meningitis when he was 18 months of age. Reduced visual acuity had been known since childhood. At 18 years of age, visual acuity was 20/40 in both eyes, and there was mild hyperopia (+1.25 dpt). The anterior segments were normal. Funduscopy showed mild central pigment epithelial irregularities in a bull's-eye pattern. Color vision testing with the Nagel anomaloscope revealed protanomaly.

At 37 years of age, the patient complained about further visual loss and progressive color vision deficiencies. The visual acuity was reduced to 20/50 in the right and 20/60 in the left eye. The fundus appearance was unchanged. Fluorescein angiography showed no further defects besides the previously known mild bull's-eye pattern. The visual fields tested with the Tübingen Automatic Perimeter showed relative and absolute central scotomas within 30° in the
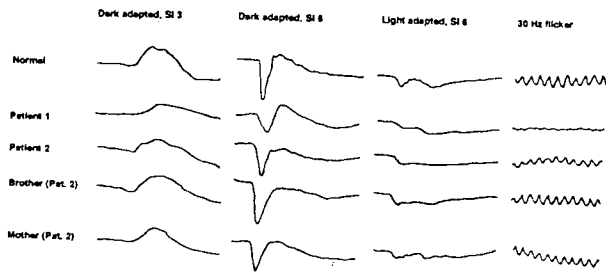
FIGURE 1. Electroretinographic recordings of white stimuli of a normal subject, patient 1, patient 2, and the mother and brother of patient 2 (identified in the front of the row of recordings). (*outer left column*) Recordings when dark adapted at stimulus intensity (SI) 3. (*middle left column*) Recordings when dark adapted at SI 6. (*middle right column*) Recordings at light adaptation. (*outer right column*) Recordings at 30-Hz flicker stimulation. Vertical calibration marks indicate 100 μV; horizontal marks indicate 50 msec for flicker stimulation and 20 msec for all other responses.

upper half of the visual field. Three years later, the clinical findings were unchanged. Electrophysiologic examinations were performed when he was 37 and 40 years of age, and psychophysical examination was performed when he was 40 years of age.

The patient's brother is 5 years older and was examined when he was 42 years of age. Visual acuity was 20/20 in the right eye. The left eye had reduced visual acuity, 20/500, because of amblyopia ex anisometropia (right eye, ±0.0 dpt; left eye, +7.75 dpt). Fundus examination and visual field testing showed normal findings.

The mother of the two brothers was examined when she was 66 years of age. Her visual acuity was 20/30, and she had hyperopia of +7.0 dpt in both eyes. The anterior segment showed a senile cataract. There were slight pigment irregularities at the posterior pole, but they were not in a bull's-eye pattern. Visual fields were normal.

Electrophysiologic Findings

Electrooculography. Electrooculography was not performed in patient 1. The light rise was similarly reduced in patient 2, his mother, and his brother to approximately 125% to 139%. Our normal values are 187% ± 36% (±2 SD; lower normal range, 151%).

Standard Electroretinography. Standard electroretinography was performed in patients 1 and 2 and both family members of patient 2 (Fig. 1). Part of the results obtained for patient 2 and his brother have been described previously.[13] When dark adapted, the a- and b-wave amplitudes were subnormal for patient 1. This was attributed to high myopia and not to rod dysfunction because of the normal dark adaptation. In patient

2, the a-wave amplitudes were normal, and the b-wave amplitudes were reduced. The brother and mother of patient 2 showed normal a-wave amplitudes and subnormal b-wave amplitudes.

At light adaptation, the a-wave amplitudes were subnormal in patient 1 and borderline in patient 2. The b-wave amplitudes were severely reduced in both patients. The brother and mother of patient 2 had normal a-wave amplitudes and reduced b-wave amplitudes. The amplitudes of the 30-Hz flicker response were reduced in both patients and normal in the family members of patient 2.

Color Electroretinography. Electroretinograms to chromatic stimuli were recorded in patients 1 and 2. Color electroretinographic results for patient 2 have been described previously.[16] When dark adapted, the a- and b-wave amplitudes were reduced in patient 1. In patient 2, the a-wave amplitudes were reduced to red stimuli and normal to all other stimuli. The b-wave amplitudes were borderline for green stimuli and reduced for the other chromatic stimuli. In both patients, the b-wave implicit times were normal for all except red stimuli, when they showed marked prolongation.

When light adapted in both patients, no measurable responses were recorded for red stimuli (Fig. 2). In patient 1, the a-wave amplitudes were subnormal for blue and blue–green, reduced to green, and severely reduced to yellow stimuli. In patient 2, the a-wave amplitudes were borderline for blue, blue–green, and green, and they were reduced for yellow stimuli. B-wave amplitudes were not measurable for either patient.

In patient 1, 30-Hz flicker stimuli elicited reduced responses for blue–green, green, and yellow stimuli,
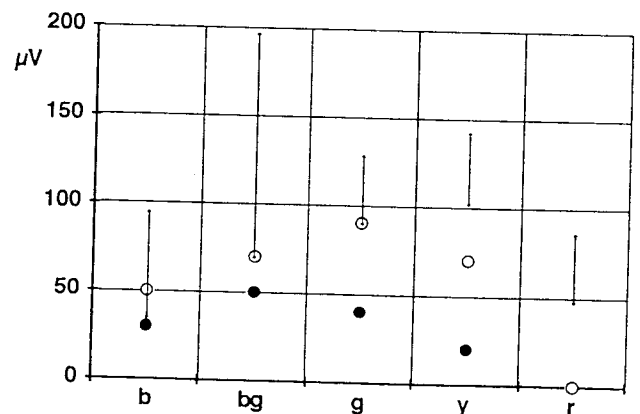

FIGURE 2. Electroretinographic recordings of blue (b), blue–green (bg), green (g), yellow (y), and red (r) stimuli. A-wave amplitudes at maximum light intensity under white light adaptation of patient 1 (*filled circles*) and patient 2 (*open circles*). The vertical lines indicate the limits of the normal range (±2 SD).

but there was no response for red stimuli. In patient 2, responses for all chromatic stimuli were reduced, but the reduction was most distinct for red stimuli. Oscillatory potentials were missing for red stimuli and reduced for all other colors. Pattern electroretinograms were nonrecordable in both patients.

Psychophysic Findings

Color Vision. *Patient 1.* Testing with the Nagel anomaloscope revealed matches close to those of rod monochromatism along the scotopic axis for both eyes. The Panel-D 15 test and the Farnsworth–Munsell 100 hue test (total error scores: right eye = 300; left eye = 316; normal = <134) gave consistent results and showed a protan axis of confusion.

Patient 2. Color vision testing with the Nagel anomaloscope revealed a protanomaly (Rayleigh equation: 0.1) in both eyes at 18 years of age. At 37 years of age, scotopic matches were found. The Panel-D-15 test revealed errors in the protan axis. The Farnsworth–Munsell 100 hue test showed an increased error score without a typical axis (total error scores: right eye = 476; left eye = 360). In the patient's mother and brother, color vision test results were normal when the same tests were used.

Dark Adaptation. Dark adaptation was examined in both patients and all family members of patient 2. Adaptation curves were biphasic, and there was a Kohlrausch kink between 7 and 9 minutes after dark adaptation was begun. Cone branches showed normal or slightly elevated thresholds within the normal range. The final rod adaptation levels were normal.

Spectral Sensitivity. Spectral sensitivity measurements were taken and transient tritanopia was examined in both patients. Spectral sensitivity during light adaptation showed a three-peaked function in normal observers (vertical lines, Fig. 3). The spectral sensitivity of the patients was normal for S and M cones. The threshold for longer wavelengths was markedly lower. The separation of the different cone systems by chromatic adaptation revealed normal sensitivity of the S and M cones (Fig. 4). There was no measurable L-cone function.

Transient Tritanopia. In normals, the threshold to detect the blue test light rises immediately after the offset (delay = 0) of the yellow adaptation light for approximately 1 logarithmic unit and reaches the threshold obtained during yellow adaptation only 1 or 2 seconds thereafter (transient tritanopia; vertical lines, Fig. 5). The threshold increase after offset of the yellow adaptation was reduced in both patients, indicating severe dysfunction of cone–cone interaction.
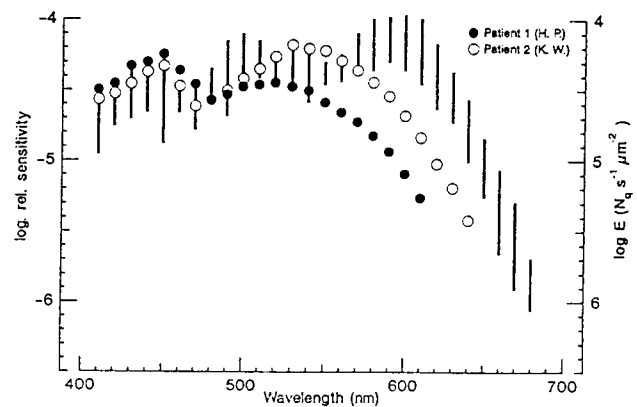

FIGURE 3. Spectral sensitivity function during light adaptation. The logarithm of the energy (E, right hand ordinate) necessary to evoke a threshold sensation at the different monochromatic wavelengths is plotted against wavelength. Normal observers show a three-peaked function, and vertical lines indicate the normal range. The spectral sensitivity in patient 1 (*filled circles*) and patient 2 (*open circles*) were approximately normal for S and M cones. They showed no responses to longer wavelengths.

Molecular Genetic Examinations

Molecular genetic examinations were performed in both patients. In patient 1, the L- and M-cone pigment gene array consisted of a single hybrid L–M cone pigment gene. The complete coding sequence of this gene had been determined and was normal. Patient 2 showed a protan genotype by Southern blot analysis. A hybrid L–M cone pigment gene replaced the L-cone pigment gene.

Molecular genetic examination was repeated with identical results in patient 2, and blood samples of the family members were analyzed. Southern blot analyses revealed that in the brother and mother of patient 2, L- and M-cone pigment gene arrays were normal.

DISCUSSION

Our patients obviously have progressive cone dystrophy. The slow progressive visual loss, photophobia, central scotomas, and color vision defects are typical clinical findings.[1-3,19,20] The normal course of dark adaptation revealed normal rod function. The normal to subnormal electroretinographic responses to dark adaptation and the severely reduced responses to light adaptation and to 30-Hz flicker indicate severe dysfunction of the cone systems. However, in cone dystrophy, reduced responses to all chromatic stimuli are expected,[16] as are reduced spectral sensitivity for all three cone types.[20] In our patients, the L cones—either electroretinographically or psychophysically—were predominantly affected. Molecular genetic examination revealed an alteration of the L–M cone

FIGURE 4. Spectral sensitivity functions during chromatic adaptation. Vertical lines indicate the normal range. In patient 1 (*filled circles*) and patient 2 (*open circles*), sensitivity was normal for S (*left*) and M cones (*middle*). L-cone responses (*right*) were not measurable.
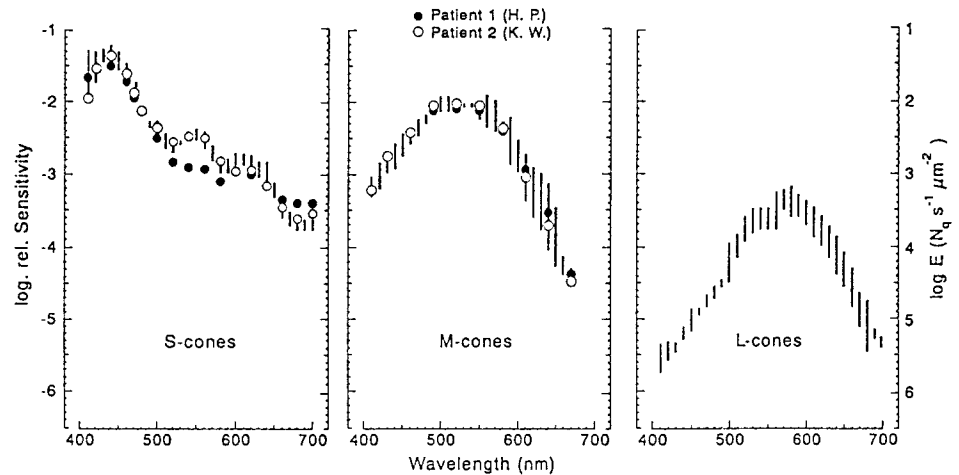
pigment gene array. In patient 1, there was only one single hybrid L–M cone pigment gene. In patient 2, there was a normal M-cone pigment gene and a hybrid L–M cone pigment gene. To date, hybrid cone pigment genes have been reported in inherited color vision defects[21,22] but not in progressive cone dystrophies. Two hypotheses may explain the findings in our patients: First, the alteration of the cone pigment genes may have induced progressive selective cone dystrophy. Second, we may have detected protanopes in whom progressive cone dystrophy also developed.

Cone pigment genes are candidate genes for the induction of cone dystrophies similar to rhodopsin gene defects in autosomal dominant retinitis pigmentosa.[23,24] Reichel et al[4] recently described an X-linked cone dystrophy with predominant L-cone dysfunction. In their pedigree, older men were affected more severely than younger men. In some women,

protan color vision dysfunction and abnormalities in the electroretinogram could be detected. The reduced responses to 30-Hz flicker and to longer wavelength stimuli are similar to findings in our patients. Reichel et al,[1] however, did not examine cone function with single flashes, nor did they perform electrooculography. Therefore, it cannot be judged whether their patients had the severely reduced cone b-waves and the same reduction of the electrooculogram light rise. Rather, in their patients, they found a deletion in the L-cone pigment gene and a normal M-cone pigment gene.

In contrast to normal or subnormal a-waves, the severely reduced b-waves are not explained easily by the rearrangement of the X-chromosome pigment gene array. They may indicate a retinal transmission defect in the cone pathway, as has been suggested.[13] Few patients of progressive cone dystrophy with reduced b-waves have been described.[10–13] These patients had either normal color vision, yellow–blue defects, deuteranopia, or achromatopsia. Spectral sensitivity measurements have not been performed in these patients. Other inherited retinal diseases with reduced b-waves, such as congenital stationary night blindness[9] or X-linked congenital retinoschisis,[8,25] could be excluded in our patients by normal dark adaptation and fundus examination. Patients with X-linked retinoschisis show reduced responses to all chromatic stimuli in the color electroretinogram (Kellner, unpublished findings, 1990).

Both the mother and the brother of patient 2 had reductions in the light rise in the electrooculogram as well as, to some extent, b-wave amplitude reductions. Apparently, these functional defects were stationary in the family members. There was neither an indication for progressive disease in the general history nor a marked difference between the findings in the mother and the brother of patient 2, which might indicate
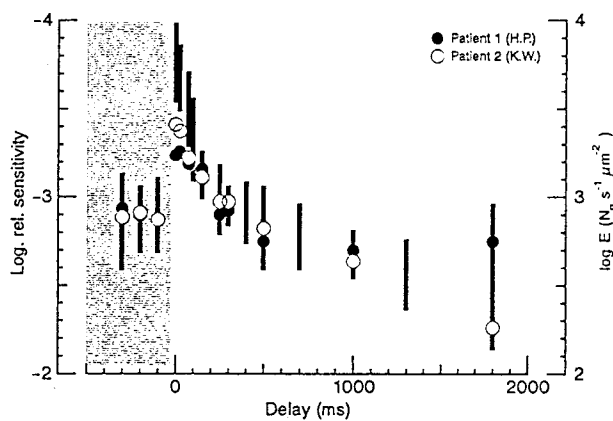

FIGURE 5. Transient tritanopia. The threshold for a blue test light increases transiently after the offset of a yellow adaptation light at $t = 0$. The normal range is indicated with vertical lines (±1 SD). In patient 1 (*filled circles*) and patient 2 (*open circles*), the transient threshold increase is reduced compared with normals.

progression with age. Molecular genetic examination revealed normal results. In this family, these findings may indicate that another retinal defect is inherited, common to all family members, whereas L-cone dysfunction is present only in patient 2.

High myopia and reduced visual acuity, as seen in patient 1, have been described in patients with nonprogressive, incomplete achromatopsia and protan luminosity function.[6] Those patients showed only rod and M-cone function, but electroretinography revealed normal a- and b-waves. Although clinical findings in patient 1 look similar to these nonprogressive forms, the visual deterioration strongly indicates progressive disease.

In patient 1, the single hybrid L–M cone pigment gene apparently leads to a cone pigment with sensitivity similar to that of a normal M-cone pigment. It cannot be excluded, however, that this rearrangement of L- and M-cone pigment genes causes slow retinal degeneration.

We have found alterations of the cone pigment gene array in our patients with cone dystrophy. At the current ages of our patients, however, we are unable to distinguish between predominant L-cone dystrophy and protanopia that develops cone dystrophy. The clinical course indicates early-onset, slow, progressive retinal degeneration in both patients. In children with protanopia, one would expect normal visual acuity, but this was not the case in our patients. The spectral sensitivity was normal for M cones in both patients, indicating that at least some M cones in the fovea functioned normally. Responses to 30-Hz flicker and green stimuli are normal in protanopia.[16] The reduced responses in our patients indicate that the dystrophy involves the M cones with a hybrid L–M cone pigment gene in patient 1 and the M cones with a normal M-cone pigment gene in patient 2. One might suspect that the rearrangement of the X-chromosome cone pigment gene array induced progressive cone dystrophy in contrast to the stationary retinal dysfunction observed in the family members with normal cone pigment genes. Examinations of similar patients with larger families are necessary to prove this theory.

Key Words

cone dystrophy, cone pigment genes, electroretinography, retinal degeneration, spectral sensitivity

Acknowledgments

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