Color Vision and Genetics in a Case of Cone Dysfunction Syndrome

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Abstract: Tests of color vision and unique hue judgments were carried out on a patient with little cone function and abnormal macular pigmentation and optic nerve appearance. These tests revealed weak cone-based dichromatic color vision yet normal red, green, and blue unique hue judgments. In addition, rod input was found to inhibit color discrimination. Genetic analysis revealed the absence of L pigment genes, but multiple M pigment genes. A mutation that inactivates the encoded pigment was identified in a subset of the M genes. One of these genes with the mutation was in the first position of the array. Thus, these visual deficits and changes in the integrity of the inner retina may be linked to defects in the photopigment genes. © 2000 John Wiley & Sons, Inc. Col Res Appl, 26, S284–S287, 2001

Key words: color vision deficiency; rod-cone interaction; optic nerve; achromatopsia

INTRODUCTION

Optic nerve and cone dystrophies along with the subclassification of cone dysfunction syndrome represent a heterogeneous population exhibiting a wide variety of symptoms.[1–3] In particular, color vision changes ranging from complete achromatopsia to mild red-green anomalies are often observed. The causes of some of these conditions are believed to be genetic in nature (e.g.,[1,4]) and the patterns of inheritance vary from one affected individual to another. The present study presents findings from a subject originally diagnosed with optic nerve dystrophy as a small child and later with cone dystrophy. Our current and more specific diagnosis is one of cone dysfunction syndrome.[2] We were interested in how our subject's condition affects his color experience. Therefore, we measured color discrimination, rod influence on color discrimination, and unique hues. We also performed electroretinograms to assess cone function, and molecular genetic analysis to investigate possible causes of the condition.

METHODS

Subject

The subject (JBN) is a 31-year-old male who had been diagnosed with optic nerve dystrophy when less than one year old by a family ophthalmologist. Another clinician for the present study made a recent independent diagnosis of "stationary congenital cone dystrophy." Our present diagnosis is "cone dysfunction syndrome."[2,3]

The subject's best-corrected visual acuities were 20/200 in both eyes. His threshold visual fields showed some areas of reduced macular sensitivity. His fundus examination showed both optic nerves with hazy, ill-defined borders. There appeared to be some optic disc elevation with no cupping. The macular showed well-circumscribed RPE depigmentation 360° around the fovea. ERG measures revealed severely reduced photopic ERGs and no recordable 30 Hz flicker responses (data not shown).

Color-vision deficiency was detected with the Ishihara Color Plates (0/12). The subject was previously unaware that he had unusual color vision and attributed most of his vision problems to his reduced visual acuity.

His pupillary reflexes were normal. His extraocular muscle movements were full and smooth in all fields of gaze. There was also some micronystagmus with slit lamp observation. A photostress recovery test appeared normal; 40 s

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(OD), 50 s (OS). The anterior segments of both eyes were normal. His intraocular pressures were normal.

Psychophysics

In addition to the Ishihara plate tests, we measured color discrimination using the Cambridge Colour Test, and measured locations of unique hues. The Cambridge Colour Test has been described in detail previously.[5] For this test, the stimuli were viewed monocularly from 195 cm with the gap in the pattern subtending 1°. The luminance of the display was 18 cd/m^2. The test was administered both with and without prior exposure to an adaptation light designed to reduce contribution from rod receptors. The adaptation originated from a cold-filtered tungsten source, had an intensity of 4×10^7 scotopic trolands and lasted 5 s. For the rod bleach conditions, the subject was readapted every 6 min. Chromatic discrimination thresholds were measured from 3 different points in color space within the isoluminant plane. Both the Cambridge Colour Test and the unique hue measurements were made using stimuli generated with a Cambridge Research Systems graphics board and presented on a Sony monitor.

Unique hues were measured for stimuli presented as a Gaussian tapered pulse (260 ms on; 250 ms off) in a centrally fixated 5° field. The stimuli had a fixed chromatic contrast of 60 units and a mean luminance of 30 cd/m^2 within a scaled version of the MacLeod–Boynton chromaticity space.[6] Hue angle was varied using two randomly interleaved staircases and a forced-choice hue judgment (e.g., too red or too green) for unique yellow.[6]

Molecular Genetics

Molecular genetic analysis was performed to determine the type and numbers of the X-linked pigment genes. Genomic DNA was isolated from peripheral blood leukocytes. Exon 5 of all of the X-chromosome cone pigment genes was amplified in the polymerase chain reaction with primers 5′tgtaaaacgacggccagtTCCAACCCCCGACTCACTATC, and 5′caggaaacagctatgaccACGGTATTTTGAGTGGGATCTGCT, which correspond to sequences within introns 4 and 5, respectively.[7] The lowercase letters indicate the M13-21mer and M13R primer sequences, which tag the opsin gene primers (uppercase letters). The thermal cycling conditions were 95°C for 9 min, followed by 30 cycles of 94°C for 45 s, 59°C for 45 s, and 72°C for 45 s. Both strands of the PCR product were used directly in DNA sequencing using the dye-primer sequencing kit from Perkin–Elmer.

Exon 4 was amplified from all genes in the array using a primer corresponding to the 5′ end of exon 4 and to intron 4 near the 3′ end of exon 4. These primers span the region encoding amino acid 203. The forward and reverse primer sequences were 5′tgtaaaacgacggccagtCCCACGGCCTGAAGACTTC and 5′caggaaacagctatgaccGGAAAAATAGTTTAGGAGTCTCAGTGGA, respectively. The lowercase letters indicate the M13 primer tags used for sequencing. The uppercase letters correspond to the opsin gene sequences. In addition to analyzing all exons 4 and 5, the first gene in the array was individually amplified, and directly sequenced as previously described.[8]

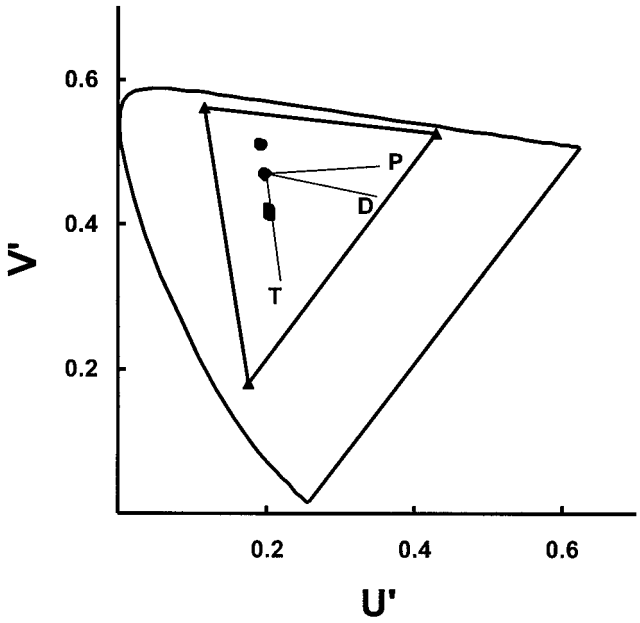

FIG. 1. Chromatic discrimination contours for a normal trichromat measured with the Cambridge Colour Test and plotted in CIE LUV color space. The gamut of possible colors determined by the monitor phosphors are shown by the large triangle. Discrimination thresholds were measured from three reference points located at the centers of the small clusters of data points. The protan (P), deutan (D), and tritan (T) confusion axes originating from the central reference point are indicated. Best-fitting ellipses are also included, but are too small to be discerned at this scale.

RESULTS

Chromatic Discrimination

Figure 1 shows chromatic discrimination contours at three reference chromaticities for a normal control subject based on the Cambridge Colour Test. The data are plotted in CIE LUV space. As expected for normal trichromatic vision, the discrimination ellipses for this subject are quite small and cluster tightly about the three reference points. These data can be contrasted with subject JBN's chromatic discrimination ellipses shown in Fig. 2, which are much larger than the control's but more weakly oriented than typical protan or deutan ellipses. These results thus reveal a profound and fairly general loss of color discrimination.

Discrimination thresholds for subject JBN measured after a strong rod bleach are shown in Fig. 3. Interestingly, thresholds measured after the rod bleach are much improved over the unadapted condition for discriminations along the tritan direction, suggesting suppressive rod influence on his chromatic sensitivity.

Unique Hues

Unique hue settings are shown in Fig. 4 within a scaled version of the MacLeod–Boynton color space.[6] JBN's indi-

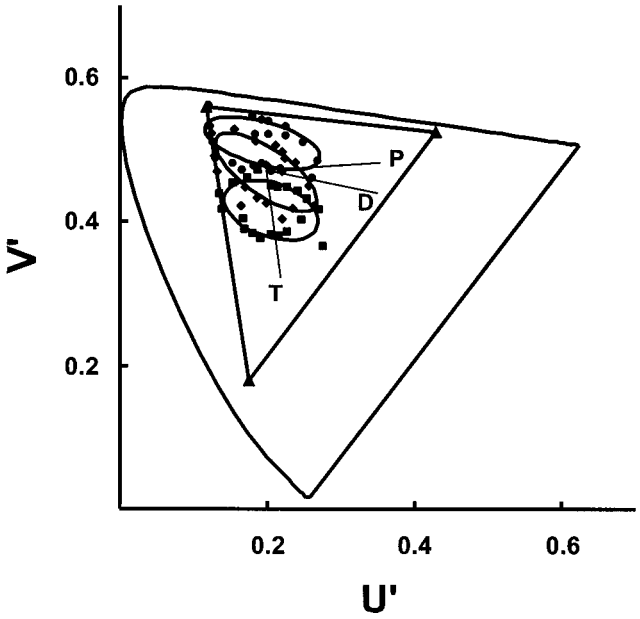

FIG. 2. Chromatic discrimination contours for subject JN. Other details as in Fig. 1.

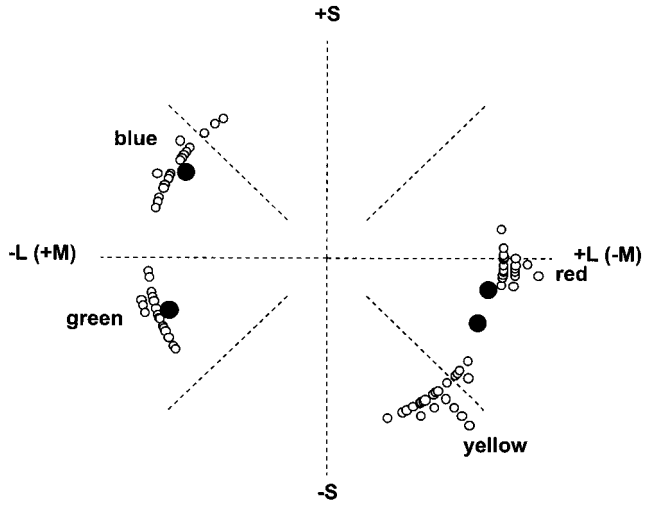

FIG. 4. Unique hue judgments plotted in the scaled MacLeod–Boynton color space. The S and LM cardinal axes are indicated. The ranges of unique hue settings from normal trichromats are shown by the solid arcs. Unique hue judgments from subject JN are shown by the crosses and open triangles.

vidual unique hue settings are shown by the symbols, while the solid arcs show the range for a group of normal trichromats (tested with 2° fields).[9] Despite poor performance on the discrimination task, JBN makes unique hue settings of blue and green that fall within the range of the normals. JBN's unique yellow and red settings fall slightly outside the normal range.

Molecular Genetics

Analysis of the X-linked pigment gene arrays revealed possible causes of the loss of cone function in JBN. DNA sequencing of exon 5 revealed that subject JBN does not possess a gene that encodes for long wavelength (L) photopigment. In this regard, JBN is an obligate protan. In addition, direct fluorescent DNA sequencing revealed a missense mutation in a subset of the M genes for JBN. The mutation substituted arginine for cysteine at position 203 (C203R). This cysteine is essential for pigment function.[10] This same deleterious mutation was found previously in individuals with blue-cone monochromacy[11] and red-green color deficiencies.[12] Mutation of the corresponding cysteine in the rod pigment, rhodopsin (C197Y) results in a very severe form of autosomal dominant retinitis pigmentosa (adRP).[13] For JBN, the mutation was present in the photopigment gene positioned first (most upstream) and it was present in at least one of the downstream M photopigment genes. JBN also had additional M genes that did not have the deleterious mutation. Because the mutation inactivates the pigment, all the cones that exclusively express mutant pigment would be nonfunctional. In summary, JBN has two separate alterations in his X-encoded cone pigment genes. His normal L pigment gene(s) have been deleted, and he has mutations that inactivate the photopigments encoded by two of the remaining M pigment genes. These results suggest that JBN's vision disorder, including the symptoms of poor color discrimination and acuity, and inner retinal changes may be due to the combined effects of the deletion and point mutations in the X-chromosome photopigment gene array.

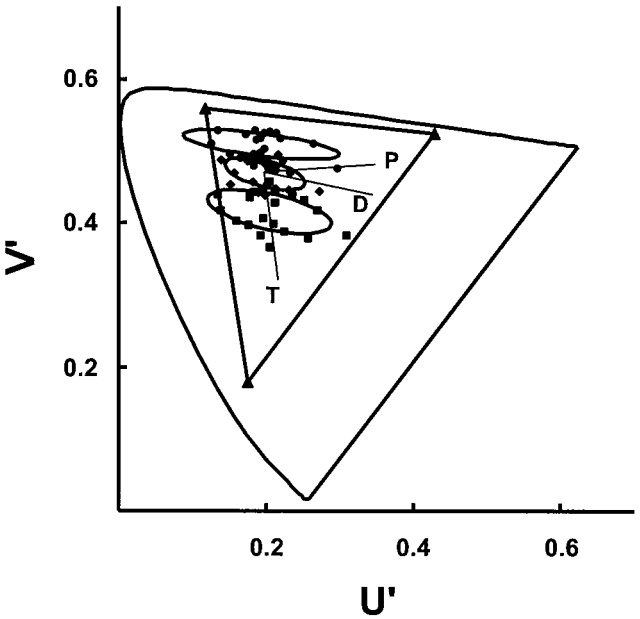

FIG. 3. Chromatic discrimination contours from subject JN after exposure to an adapting stimulus designed to desensitize the rods. Other details as in Fig. 1.

DISCUSSION

The results from measurements of chromatic discrimination suggest a general loss of color vision and a classification as incomplete achromatopsia. There is some indication of weak color vision that improves after adaptation designed to desensitize rods. Despite these profound losses, subject JBN

is able to make normal green and blue unique hue judgments. In addition, the variability in JBN's unique hue settings is slightly less than found in a sample of normal subjects.[9] The above data suggest that unique hue judgments are not strongly constrained by chromatic sensitivity (see also[14]).

The genetic analyses reveal that subject JBN has a rare point mutation in the first gene in the array, which renders the encoded photopigment nonfunctional. In addition, he has lost all L pigment genes, as is typical in individuals with protan color-vision defects. The loss of L pigment genes accounts for his reduced red-green discrimination as revealed on the Ishihara plate tests and the red-green orientation of the discrimination ellipses.

The C203R mutation was present in the first gene of JBN's array. Among men with normal color vision, 50–90% of the cones in the central retina express the first gene in the array.[15] Thus, owing to the mutation in the first gene, JBN may express the C203R mutation in 50–70% of his cones, rendering them nonfunctional. Any cones that express the downstream mutant M would also be rendered nonfunctional. A paucity of functional M cones might help explain his poor blue-yellow discrimination as well as the loss of visual acuity. However, subject JBN also has copies of the M gene that do not encode the C203R substitution. It is notable that, while these presumably normal M pigment genes do not prevent severe loss of acuity and an abnormal fundus appearance for JBN, his results would be consistent with some M cone function owing to these genes.

It is also of interest that JBN's tritan chromatic discrimination improves after desensitizing the rods with an adaptation light. Previous research has shown similar suppressive input from rods to chromatic discrimination in normal human trichromats (e.g.[16–18]). However, in color-deficient individuals including blue-cone monochromats, the presence of rod input usually facilitates chromatic discrimination. The improvement in discrimination thresholds after a rod bleach in subject JBN agrees with his subjective report of an increase in the perceived saturation of colors after rod adaptation and under bright ambient illumination. It is of note that, although colors appear more saturated under bright light for subject JBN, he still prefers dimmer lighting conditions for most tasks.

As noted above, subject JBN was originally diagnosed with optic nerve dystrophy rather than cone dysfunction syndrome because of low visual acuity and an abnormal fundus appearance. As noted previously,[2] failure to recognize this condition as a cone dysfunction syndrome is common. It is, therefore, of interest that in the present case the condition appears to stem from deleterious mutations in photopigment genes and does not originate primarily in the inner retina. This suggests the possibility of a link between defects in the photopigment gene array and conditions believed to be primarily of inner retinal origin and, thus, the integrity of the inner retina may be compromised by defects that originate in the cones. The frequency with which inner retinal changes are associated with defective pigment gene arrays has yet to be determined.

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