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Visual Pigment Gene Structure and the Severity of Color Vision Defects

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Rearrangements of the visual pigment genes are associated with defective color vision and with differences between types of red-green color blindness. Among individuals within the most common category of defective color vision, deuteranomaly, there is a large variation in the severity of color vision loss. An examination of specific photopigment gene sites responsible for tuning phototipigment absorption spectra revealed differences that predict these variations in the color defect. The results indicate that the severity of the defect in deuteranomalous color vision depends on the degree of similarity among the residual photopigments that serve vision in the color-anomalous eye.

Predicting the severity of a deficit from examination of a person’s genetic makeup may prove to be particularly challenging for disorders that involve the nervous system and manifest themselves primarily as differences in behavior. Nonetheless, here is an example: a class of human color vision defect in which differences among the genes predict the severity of color vision loss. Deuteranomaly is the most common inherited color vision defect, affecting more than 1 in every 20 men in the United States. The condition arises from the absence of one of the cone photopigments, the normal pigment that is sensitive to middle wavelengths (M). Even though deuteranomalous individuals are missing normal M photopigment function, they retain varying degrees of trichromatic color vision, which is based on a pigment that is sensitive to short wavelengths plus two narrowly separated photopigments that absorb in the long-wavelength (L) region of the spectrum.

In this study, 16 young men were identified as deuteranomalous on the basis of a standard color matching test for red-green color vision defects—the Rayleigh match.

In this test, the person is asked to mix together a red and a green light in a proportion that exactly matches the appearance of a monochromatic yellow light, as has been previously described (1). A deuteranomalous person chooses a much higher proportion of green light in the mixture than does a person with normal color vision. This color-matching test also distinguishes deuteranomalous trichromats from dichromats (as was done in (2)), who have only two cone photopigments and thus suffer the most severe of the common red-green color defects. Once we differentiated deuteranomalous men from men with normal color vision and from dichromats by means of the Rayleigh match, we assessed the severity of the color vision impairment using the American Optical, Hardy, Rand and Rittler (AO-HRR) pseudoisochromatic plates for color vision testing. From the complete set of plates included in the test, we used a series of six test figures designed for grading deutan color defects (Fig. 1A). Each design in this sequence is composed of a reddish-colored symbol on a background of gray dots. Each symbol in the progression is more intensely colored than the last.

The deuteranomalous participants varied enormously in their performance on this test. Although all 16 men had been classified as deuteranomalous in the color-matching test, a subset of these participants...
nonetheless behaved normally in the everyday tasks of recognizing, naming, and sorting colors. This subgroup reported that they had no indication of a color vision abnormality before being identified as "color deficient" by formal color vision testing. When given the plate test, individuals in this group were challenged only by the most difficult plate (plate 1, Fig. 1A), a plate that some people with normal color vision also fail to interpret correctly. In contrast to the least affected men, the most extreme case (participant 189) was unable to detect any of the symbols, even the one with the largest color difference from its background (plate 6, Fig. 1A). The symbol, an "X", in that design, appears to be red to those with normal color vision, but it was invisible among the gray dots to participant 189.

The extent of the defect can be expressed numerically as the distance (D) in color space that must differentiate the symbol on the plate from its background before the person can interpret it correctly (3, 4).

Fig. 1. (A) Reproductions of five symbols from the AO-HRR pseudochromatic plates used to assess the degree of color vision defect. In order of decreasing difficulty from left to right, they are read as R, O, D, X by a person with normal color vision. The numbers below each plate are the D values (3) used to quantitatively express the color difference between the symbol and its gray background. One plate (no. 5 in the progression) is not shown. Plates are shown as examples of points discussed in the text and not for color vision testing. The AO-HRR plates are printed with permission from Richmond International Incorporated (copyright 1954). (B) Spectral tuning of L pigments in deutanomalous men. The majority of the spectral difference between L and M pigments is specified by exon 5. The red arrowhead indicates exon 5 of an L pigment gene. Exons 2 through 4 encode amino acid substitutions that produce relatively smaller spectral shifts among the L pigments. In the diagrams of the genes (arrows) shown here and in (C), except where specifically noted, red and green denote codons specifying the amino acids indicated in the diagrams below the arrow at left in (B). Red: T, threonine; S, serine; I, isoleucine, and A, alanine. Green: I, isoleucine; V, valine, Y, tyrosine, A, alanine; T, threonine; and S, serine. To predict the spectral separation of the pigments in deutanomalous men, the table shown at right was used. The absorption maxima ($\lambda_{max}$) of the L pigments encoded by the diagrammed genes were measured in vitro (15, 16). (C) Analysis of the X-linked visual pigment genes in deutanomalous men. The second column from the left shows the gene arrangements as deduced from Southern analysis (23). The number of M-L hybrid genes and the number of M pigment genes are each indicated by a subscript number or by n if the number was not determined. Southern analysis does not give information about the fine structure of the genes. In the present experiments, the gene furthest upstream (first gene) and one downstream hybrid M-L pigment genes were analyzed separately (17, 18). The deduced structures of the first gene and the downstream hybrid M-L pigment genes are shown. Except for the first gene, shown placed at the left end of each array, which was always observed to have an L gene exon 5, the relative order of the genes is not known. Exons 2, 3, and 4 were examined to determine whether the first gene and the downstream hybrid M-L pigment genes differed at sequences in exons 2, 3, and 4 that encode spectrally active amino acid loci (17-19). We have included for comparison the results from two dichromats (deutanopes; top two rows) from a previous study (2). The asterisk indicates individuals who had sequences representing multiple hybrid M-L pigment genes with subtle differences in exon 2; sequences that specified ile$^{65}$, val$^{111}$, and tyr$^{116}$ were present but so were exon 2 sequences that specified one or more of the amino acids thr$^{65}$, ile$^{111}$, and ser$^{116}$, An issue for the three participants (028, 190, 017) who have multiple fusion genes that are substantially different is that there is evidence to suggest that not all the distal genes in the array are expressed (25). For these men, it is possible that not knowing which gene is expressed could limit predictive power. In the absence of information about which genes are expressed, the spectral separation values given are the largest possible for the L and M-L pigment combinations each man could have, based on the deduced gene structures. Participants 017 and 190 would be predicted to have a spectral separation of 9.9 nm if they expressed only the fusion gene that is least different from the first gene in the array. This would not substantially change their predicted behavior. The worst case for participant 028 would predict a 3.7- to 4.0-nm spectral separation and poorer color vision than he exhibits. Spectral separations were calculated with the $\lambda_{max}$ values measured in vitro (values from (15) are indicated by †; values from (16) are indicated by ‡) shown in Fig. 1B.
Lower D values indicate better color vision; the men categorized as having "very mild" defects were able to read the plate with D = 0.022. Higher D values indicate poorer color vision; participant 189 was assigned D > 0.081 because he was unable to interpret even the most vivid plate.

Results from Southern (DNA) analysis have revealed that the pigment gene arrangements of deuteranomalous men are different from those of most men with normal color vision (5-7). However, even though deuteranomalous people vary in the numbers and ratios of their M and L cone pigment genes, as do normals (5, 6, 8), the genetic differences failed to correlate with the extent of color vision defect. Southern analysis of the pigment genes of our 16 participants also showed no correlation with differing degrees of color vision defect (Fig. 1C). It is noteworthy that most of the deuteranomalous men had apparently complete M pigment genes. These are indicated by the green arrows in Fig. 1C. The reason M pigment genes do not contribute to deuteranomalous color vision is not understood.

In people with normal color vision, the capacity to distinguish colors in the red-to-green region of the spectrum is based on the difference between the M and L cone pigments. In contrast, the color vision of the deuteranomalous is based on two more narrowly separated pigments (9-11). One hypothesis that has been proposed to explain the differences in color vision among deuteranomalous people is that individuals differ in the spectral separation of their remaining X-encoded pigments. A deuteranomalous person who has well-separated pigments would have better color vision, whereas one whose pigments are more similar would have poorer color vision. This idea has been called the "spectral proximity hypothesis" (4). A molecular genetic test of the spectral proximity hypothesis requires information about the amino acids that control the spectral sensitivities of the X-encoded visual pigments; information that is now available (2, 12-16). All spectral differences are encoded by exons 2 to 5. The largest spectral shifts are encoded by changes in exon 5 that divide the X-encoded pigments into two major classes, M and L (9, 13). Exons 2, 3, and 4 each encode changes that produce relatively smaller spectral shifts, making them candidates for controlling spectral differences among the pigments in deuteranomalous people (Fig. 1B).

We studied the genes of the 16 deuteranomalous participants by performing long polymerase chain reaction (PCR) and then examining the products by means of restriction analysis and direct sequencing (17-19) to analyze the X-linked pigment gene array of each man (Fig. 1C). We then constructed a model of the pigment gene arrangement for each person that was used to predict the largest spectral separation among his L pigment. On the basis of color vision behavior, participants segregated into five different levels of deutan color vision defect, ranging from dichromatic to nearly normal.

Spectral tuning studies suggest that amino acid differences encoded by exons 2, 3, and 4 of the photopigment genes are most likely to be responsible for the spectral separation between the X-encoded pigments underlying deuteranomaly. The spectral proximity hypothesis, supported by our results, predicts that the presence of the active differences in all three exons would be required to produce the largest spectral separation and therefore the best deuteranomalous color vision. The presence of relatively fewer active differences would predict poorer color vision. Most of the participants who were least affected (very mild) had differences in all three exons among their genes with an L pigment exon 5. Those who were the second least affected (mild) had differences in exon 2 plus differences in either exon 3 or 4, but not both. The severely affected men had differences in exon 2 but in neither exon 3 nor 4. These people were distinguished from the dichromats who were identified as having a single gene sequence. A few men did not follow this trend but were nonetheless predicted from the DNA sequences. For example, participant 182 has quite good color vision but he has differences in the same set of exons as do participants 191, 185, and 027, who have poorer color vision. This is explained by the observation (16) that differences encoded by exons 2 and 4 have a larger effect when they occur in pigments with Ser400 (as in participant 182) than when the pigments share Ala400. The general correspondence between the spectral separation predicted from the genes and the severity of vision defect supports the spectral proximity hypothesis.

The spectral proximity hypothesis has often been challenged and alternate hypotheses proposed. For example, it has been suggested that differences in the severity of color vision defects derive from variation in the amount of pigment produced (11, 20) or in the stability, quantum efficiency, or signaling of the pigment molecule (4), or in some neural factor that controls the chromatic signal at a level beyond that of the photoreceptors (21). The original version of the spectral proximity hypothesis failed to reliably predict behavior, not because the concept was wrong but because it wrongly assumed that there was a fixed L pigment common to all normal and deuteranomalous people alike (22). In truth, relative shifts among the pigments are what account for differences in behavior. For example, participant 189 had the poorest color vision of all the deuteranomalous men, yet he has differences in both exons 2 and 3 as does participant 028, a person with only a mild defect (Fig. 1C). In participant 028, the functional changes between two genes are segregated, so that the sequences that specify spectral shifts toward the red are assembled in one gene and those that shift it toward the green are in another gene. These yield pigments with a spectral separation of about 8 nm. In contrast, participant 189 has the long-wave redward-shifting exon 2 in his first gene but it is paired with the green-shifting exon 3. The long-wave exon 3 is in his downstream genes. The pigments produced by this gene combination are close in spectral sensitivity, hence his very poor color vision.

REFERENCES AND NOTES

3. The degree of color vision defect can be expressed quantitatively from measurements of the colors in the designs as specified by their coordinates, in this case in units of the Commission International de l’Eclairage (CIE) u’v’ diagram following KE. For normal color vision, this represents an approximation of a two-dimensional color diagram in which equal distances in different locations correspond to equal perceptual differences. Thus, the extent of the defect can be expressed numerically as the distance (D) in the color diagram that must differentiate the symbol from its background before the person can interpret it correctly.
17. The Expand Long Tail PCR System (Boehringer Mannheim) was used exactly as recommended by the manufacturer to do long PCR. The first gene in the array was amplified in long PCR with primers 5’-AGGCGACGGCTACCGGAGT and 5’-ACGTTAT-TTGGATGTTAGCTGTC, which correspond to se
quences 862 base pairs (bp) upstream of the first exon of the first gene (23) and to the 3' end of intron 5 (8), respectively. The PCR product served as a template to amplify exons 2, 3, 4, and 5 as described previously (2, 8, 24).

18. Restriction analysis was done on exons 3, 4, and 5 from the first gene and on exons 3 and 4 from downstream L genes. The presence of an Rsa I site in exon 5 from the first gene from each male identified it as a L pigment gene (8). Whether exon 3 contains a Bso PI restriction site or not indicates whether codon 186 specifies alanine or serine, respectively (24). A Dde I site is found in exon 4 only when serine is specified by codon 233, and this was used to deduce whether the first gene or the downstream L genes were M4LS hybrids. The results of the restriction analyses were confirmed by direct sequence analysis (24), which was also used to determine whether the sequence of exon 2 differed among the L pigment genes in individual men.

19. For men whose L pigment genes did not differ in exon 4 (Fig. 1C), all L genes except the first gene in the array were amplified by long-PCR (17) with primers 5'TTAGGACCTGGTCGGGAAG and 5'CATGATGATGAGTGTTGGAT, which correspond to sequences 466 bp upstream of the first exon of all genes in the array except the first one (5), and to L gene-specific exon 4 sequences (24), respectively. For men whose L genes differed in exon 4 (Fig. 1C), no PCR product was obtained with this primer pair, presumably because their downstream L genes were hybrid genes with an M gene exon 4 sequence. For these men, downstream L pigment genes were selectively amplified by long-PCR (17) with the use of an exon 2 primer (5'-CCTGGAAGGCGGAATTA) paired with a L gene-specific exon 5 primer (24). The PCR product was used to amplify only the downstream L pigment genes with the use of the same exon 2 primer and an M gene-specific exon 4 primer (24).

22. An exception, however, is the model, prospective for its time, forwarded by M. Alpern and J. Moeller who proposed variation in normal L pigments [J. Physiol. (London) 266, 647 (1977)].
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